
National Institute of Neurological
and Communicative Disorders
and Stroke

Intramural Research



Annual Report
Fiscal Year 1987

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

National Institute of Neurological
and Communicative Disorders
and Stroke

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U.S. DEPARTMENT
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October 1, 1986 through September 30, 1987
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Director, Division of Intramural Research

Irwin J. Kopin, M.D.

In FY 1987 the Intramural Research Program was retitled the Division of Intramural Research. The Division of Intramural Research (DIR) in the National Institute of Neurological and Communicative Disorders and Stroke conducts investigations in a wide array of disciplines related to the neurosciences in clinics and laboratories located mainly on the Bethesda campus of the National Institutes of Health. Laboratory components at Rockville and Fort Detrick in Maryland, and the Marine Biological Laboratory (MBL) at Woods Hole, MA, also make important contributions to the research efforts. Federal Government scientists, with their support staff, guest researchers and volunteer workers, continue to make important new discoveries which have significant impact on the explosive growth of knowledge about the nervous system and its disorders. Research is initiated by scientists with interests which range from the fundamental basis for molecular interactions which regulate growth, development, function, and mechanisms or drug action on the nervous system- -to clinical relevant developments of new diagnostic and therapeutic procedures targeted on the prevention, retardation, cure, and symptomatic control of neurological and communicative disorders. The results of these investigations contribute to the advance of many aspects of biomedical knowledge and contribute particularly towards the main mission of the Institute and of NIH which is the prevention or alleviation of human suffering from disease or injury. The important accomplishments and the status of major advancing inroads towards understanding neuronal function and dysfunction are summarized in subsequent sections of this report. This portion will address primarily issues which have a major impact on the administration and resources of the Institute: personnel, financing, and available space. The management of the Division of Intramural Research is a team effort which involves the active participation of Dr. Mark Hallett, Associate Director for Branches (who is also Clinical Director, NINCDS) ; Dr. Ernst Freese, Associate Director of Laboratories; and

the Director. Their administrative skills, scientific expertise, wise counsel, along with able administrative officers, have contributed immensely to the smooth operation of the Division and through resource allocation, the evolution of the direction of the intramural research.

Personnel

During FY 1987 the DIR has exploited fully its employment ceiling by hiring young investigators and expanding research efforts in support of AIDS research, and anticipating allocation of additional full-time equivalent (FTE) resources which have been requested. As FY 1987 draws to a close, there are over 500 employees accounting for 491 full-time equivalent FTE positions. These exceed slightly the DIR allocation of FTEs. There are 166 professional non-tenured employees (20 medical staff fellows, 45 staff fellows, 43 senior staff fellows, 16 special experts, and 42 visiting associates/visiting scientists). Although visiting fellows (VF) and Intramural Research Training Associates (IRTA) appointees (currently a total of 83) do not count against the FTE ceiling, the increase in the number of such ceiling-exempt appointments at NIH has prompted imposition of a limit on the total number of VF and IRTA positions which can be hired by each Institute. Although the DIR had planned to appoint a total of 96 in FY 1988, only 71 selections will be permitted. The use of National Research Council fellows (funds will be available to support 8-12 in FY 1988) may alleviate in part some of the problem, but it will be impossible to avoid completely a reduction in the anticipated number of ceiling-exempt appointments. Each branch/laboratory will be allocated (using an algorithm) a ceiling based on prior use of VF and IRTA positions (weighted 60%) and availability of appropriate scientific supervisors, i.e., number of tenured scientists and senior non-tenured scientists (weighted 40%). Guest workers, volunteers and IPA appointments will remain ceiling exempt. New initiatives in research related to AIDS and the nervous system will require additional personnel. We hope that the requested FTE positions will become available.

There have been several important personnel changes during FY 1987. The tragic death of Dr. Bruce Schoenberg, Chief of the Neuroepidemiology Branch, was a great personal loss to his friends and colleagues at the NINCDS, creating a void in

neuroepidemiological research throughout the world. Dr. Schoenberg was a dedicated and productive neuroepidemiologist who was throughout the world respected for his contributions to the methodology of neuroepidemiologic investigations. Dr. Schoenberg was devoted to his research in neuroepidemiology, even when critically ill; he will be remembered by the Institute and his collaborators around the world as a pioneer in research in neuroepidemiology. The selection process for a successor chief is in process, and we hope to fill this position early in FY 1988.

Dr. Roger Porter left the Medical Neurology Branch (MNB) , DIR, to assume the position of Deputy Director, NINCDS; we were pleased with this advance in his career. Although the loss will be felt, Dr. Porter will continue to be involved in the research programs. Dr. William Theodore is ably continuing the research program in epilepsy. Dr. Mark Hallett is currently Acting Chief, MNB, and plans for the MNB are under review.

Dr. Herbert Amyx, the veterinarian for the Institute, has accepted a position in North Carolina where he has a joint appointment at Duke University and the National Institute of Environmental Health. He has been temporarily replaced by Dr. William London until a new Institute veterinarian can be appointed. This position is particularly important because of the major investment of the Institute in animals for research, particularly primates, and the NINCDS effort to continue to support the requirements of the American Association of Accreditation of Laboratory Animal Care.

Dr. Robert Lazzarini, Chief, Laboratory of Molecular Genetics, has announced that he will accept the Chairmanship of a Department of Molecular Genetics at the Mount Sinai Medical School in New York City. This major loss will necessitate a search for a new Laboratory Chief, but once again, the discrepancies in salaries between academia and government are making difficult the task of retention and recruitment of the best scientists at NIH.

Dr. Edward Oldfield has been appointed Chief of the Surgical Neurology Branch (SNB). He has instituted an evolution in the research program of the SNB consistent with new emerging opportunities for surgical interventions in degenerative

diseases using tissue implants, as well as continuing to support research on vascular malformation treatment, brain tumor chemotherapy, and pituitary tumor treatments.

There have been several additions to the professional staff of the DIR. In anticipation of the expanded research relating to the effect of AIDS on the nervous system, Dr. Marinos Dalakas was converted to a clinical service tenured position in the Office of the Clinical Director. Dr. Debra Bergstrom, who is an important support person in the research program conducted by Dr. Judith Walters of the Experimental Therapeutics Branch, was converted from Senior Staff Fellow to a tenured position in a collaborative investigation.

Dr. Thomas Reese, Chief of the Laboratory of Neurobiology, was elected to the National Academy of Sciences, and the DIR was very proud of his accomplishments.

Three other tenure actions are imminent. Dr. Christy Ludow was approved by the Board of Scientific Counselors (BSC) for a transfer from the Division of Extramural Research to the DIR as a tenured scientist. Her work on the mechanism of speech impairments in neurological diseases and the contribution of speech analysis to diagnosis of neurological deficits have made her a leader in this field. Dr. Ludow has been assigned to the Section on Human Motor Control in the MNB. Dr. Eugene Major has also been approved for conversion to tenure (from a Special Expert) by the BSC, and this action awaits OPM approval. Dr. Linda Porrino, who has been collaborating with NINCDS in the investigation of 2-deoxyglucose uptake in the brain of monkeys with MPTP-induced parkinsonism, will transfer from her present position with NIDA to DIR upon BSC approval.

Space

Availability of space is the single most important limitation in exploiting optimally new discoveries and in meeting new Institute priorities. The pressing need to meet current standards for adequate animal care continues to require renovations and moves. The plan, as outlined in the FY 1986 report, to convert Building 9 into a

temporary primate facility has been abandoned because of the cost factor, and the fact that a new wing (14D) has been designed in Building 14 for this purpose. This facility will house a portion of the new Laboratory of Neuronal Growth and Regeneration and contain space for primate surgery and animal testing. The animals are to be housed in adjacent wings (14C and 14D) by the Division of Veterinary Resources. A total of about 2500 square feet will become available for NINCDS; the remaining space will be mainly allocated to NIMH.

As indicated in the report of FY 1986, the year-round NINCDS laboratories at the MBL are returning to the NIH campus. NINCDS will retain some laboratories in Building 9 to house the Laboratory of Biophysics (LB), and a portion of Dr. Daniel Alkon's research operations in the Section of Neural Systems involving invertebrates. Space in Building 9, the Park Building, and Building 36 (the area vacated by the LB) will be used to accommodate the return of the LB/MBL operations to the NIH campus. Investigations requiring use of marine animals will continue to be performed as necessary at the MBL during the months of the year when appropriate materials are available. Renovations to allow optimal space utilization for the various moves are currently in progress. Upon completion of renovations in Building 9, all of its components will be centered in one area. The four independent sections led by Drs. Quarles, Alkon, Venter and Fishman, as rotating Laboratory Chiefs, respectively, should constitute one of our strongest laboratories and provide intellectual stimulation, as well as the economics of shared research resources.

Space shortages in Building 10 limit appropriate facilities for new laboratories to meet current commitments. Negotiations with the NIH space management authorities are in progress with the hope that some relief may be obtained in FY 1988.

Fiscal Issues

Funding for intramural research in FY 1987 increased over the amount of FY 1986 by about 6 million dollars. About half of this amount was due to increased personnel

costs, about one million towards the NIH management fund, and the remainder (plus a shift of about one million dollars from contracts to "other objects") for "other objects". Increased funding for AIDS research, increased costs for research materials, and a shift in accounting for items previously included in the management fund to cost-for-service to the Institute accounted for the increment in "other objects". The current level of support is adequate to continue the impressive research operations of the Institute and to allow for some new initiatives, but additional funds to complement an increased number of personnel working on expanding high priority areas such as AIDS, neural tissue brain implants, and gene therapy, would enhance research efforts in these areas.

4

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Office of the Clinical Director
Office of the Director, Division of Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

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Office of the Clinical Director

Mark Hallett, M.D., Clinical Director

The Office of the Clinical Director handles administrative matters, mainly relating to patient care, coordination of educational activities, and delivery of neurological services. Service functions can be divided into the EEG Laboratory, the EMG Laboratory, the Consultation Service for both Neurology and Otolaryngology, Neuromuscular Services, Neuropathology, Audiology and Paraprofessional Support Services.

The two major educational conferences are the Clinical Conference (held on Tuesday afternoon), which is aimed at the Medical Staff Fellows and typically reviews a patient in detail, and the NINCDS Grand Rounds (held on Friday afternoon). The Clinical Conference includes some attention to matters of patient care and quality assurance. The Grand Rounds continues to offer CME credit.

EEG Laboratory, Susumu Sato, M.D., Chief

Diagnostic Services:

The total number of tests performed during this reporting period decreased somewhat for both EEG examinations and evoked potential tests compared with the last reporting period. This reduction may be due to the relocation of the laboratory within this reporting period.

	<u>EEG</u>	<u>Evoked Potentials</u>
NINCDS	390	251
NINCDS OPD	229	19
NIADDK	13	14
NICHD	67	20
NIMH	164	5
NCI	34	-
NHLB	19	-
NIAID	27	7
NIA	9	5
CC	8	-
OTHER OPD	112	17
TOTAL	1,072	338

Participation in Research Activity:

The EEG Laboratory maintains close ties with the Clinical Epilepsy Section of the Medical Neurology Branch and plays an important role in evaluating epileptic patients. The laboratory is capable of recording the routine EEG simultaneously with video recording which provides invaluable information for epilepsy research. The laboratory personnel are solely responsible for planning, monitoring and analyzing the chronic subdural recordings, and the electrocorticographic recordings performed during

temporal lobectomy. The laboratory staff prepare patients for PET scanning, magnetoencephalographic recording, intracarotid sodium amytal injection (Wada test),

and sphenoidal electrode recording. The EEG laboratory personnel also prepare patients for evoked potential studies by the psychology group.

Patients with multiple sclerosis are the major source of patient referral for evoked potentials. Evoked potentials also were done in patients with Alzheimer's disease, progressive supranuclear palsy, and metabolic diseases.

The EEG Laboratory provides a training environment for a Medical Staff Fellow toward certification by the American EEG Board. The Laboratory Chief continues to serve as Associate Examiner on the EEG Board.

EMG Laboratory, Jacob Meer, M.D., Acting Chief

EMG ACTIVITIES (July 1, 1986-June 10, 1987)

Referrals	NINCDS	84
	Other Institutes	44
Collaborative study	Polymyositis	14
	AIDS	26
	Niemann Pick	7
	Erectile dysfunction	7
	Xeroderma pigmentosum	3
Protocol 84-N-203	Diagnostic dilemmas	18
	Normals	9
Urodynamic studies		5
TOTAL		217

Dr. Jacob Meer arrived at the beginning of July 1986 to serve as the Acting Chief of the EMG Laboratory for one year until the arrival of Professor Roger Gilliatt in July 1987. Three medical fellows: Dr. Bruce Geller, Dr. Randall King and Dr. Richard Dubinsky have started their training in EMG under the supervision of Dr. Meer and Dr. Hallett. During the last fiscal year the laboratory was taking referrals from our own Institute as well as other Institutes, has participated in a number of collaborative investigations and conducted some independent research.

In our collaborative studies we continued to see patients with polymyositis. Some new projects involved patients with: (a) AIDS neuropathy and myopathy, (b) erectile dysfunction, and (c) xeroderma pigmentosum. Professor Fritz Buchthal visited our laboratory in October 1986 and has helped us start a project on selective recording of the IA afferent fibers.

Under protocol 84-N-203 we have studied some patients with interesting diagnostic dilemmas such as familial hypoparathyroidism with myopathy, stiff man syndrome, chronic fatigue syndrome and more. Our laboratory is also doing urodynamic studies for patients of our Institute with bladder dysfunction.

Publication:

Robbins, JH, Meer, J, Carl, J. and Sonies, B. Late development of neurologic abnormalities in xeroderma pigmentorum, a primary neural degeneration caused by defective repair of neural DNA. (Abstract) Neurology 37 (Suppl 1): 87, 1987.

The Neurology Consultation Service consists of three neurologists: Dr. Alison Wichman, (Chief), Dr. Paula Macrae, and Dr. Marinos Dalakas. The service provides emergency and routine consultations to patients hospitalized in the Clinical Center and outpatients referred to the Ambulatory Care Research Facility. In 1986, a total of 689 patients were evaluated. From July 1986 through June 1987, 608 patients were seen. Outpatients are referred to the bi-weekly Neurology Clinic or evaluated in other specialty clinics (Oncology, Hematology, Surgery). As a part of the Consultation Service, we facilitate the performance of necessary diagnostic procedures in other departments (i.e., myelography, electromyography, head scans), and arrange neurological follow-up as indicated.

Neuromuscular Services, Marinos C. Dalakas, M.D.

Dr. Marinos Dalakas provides clinical neuromuscular evaluations and expert advice in the diagnosis and management of patients with neuromuscular symptoms referred to us by all the Institutes. He has established and directs the Laboratory of Muscle Enzyme Histochemistry to provide a state-of-the-art histochemical and immunocytochemical evaluation of muscle and nerve biopsies. He performs the muscle and nerve biopsies in the operating room using local anesthesia and the biopsied specimens are processed for a battery of histochemical reactions. Approximately 100 biopsies per year are performed and processed in his laboratory. The findings are presented to clinical conferences of the respective Institutes or informal teaching sessions that Dr. Dalakas holds for the Fellows and other interested investigators.

The Laboratory of Muscle Enzyme Histochemistry also processes and reviews several muscle biopsy specimens sent to us from outside hospitals for expert advice. The referring physicians are subsequently called and the management of a diagnosed neuromuscular disorder is discussed. If a disorder fits within one of our protocols, patients are brought in for further study. Some of the collaborative investigative studies which Dr. Dalakas is currently actively involved include studies of patients with: (a) polymyositis/dermatomyositis (Dr. Plotz) NIAMS, (b) chronic fatigue syndrome due to EBV/CMV infection (Dr. S. Strauss) NIAID, (c) cystinosis and muscle carnitine deficiency (Dr. W. Gahl) NICHD, (d) Duchenne's muscular dystrophy (Dr. Podolsky) NIAMS, and (e) acquired immunodeficiency syndrome (AIDS) (Dr. Yarchoan) NCI.

In addition to the above neuromuscular services and collaborations with other Institutes, Dr. Dalakas is performing independent research in neuromuscular disorders under approved clinical protocols and studying patients with: (a) post-polio syndrome, (b) immune polyneuropathies, (c) motor neuron disease, and (d) neuromuscular aspects of human immunodeficiency virus infection. Progress in his research activities is reported with the Infectious Diseases Branch, NINCDS.

Publications:

Dalakas, MC. Chronic idiopathic ataxic neuropathy. Ann. Neurol. 19:545-554, 1986.

Dalakas, MC. Ataxia and paraproteinemic polyneuropathy: A reply. Ann. Neurol. 22:94-95, 1987.

Dalakas, MC. How relevant are recent studies of ALS and post-polio progressive muscular atrophy patients regarding PET scanning, polio antigen/antibody, and AIDS virus. Muscle and Nerve, 9:56, 1986.

Dalakas, MC. New neuromuscular symptoms in patients with old poliomyelitis: A three year follow-up study. Eur. Neurol. 25:381-387, 1986.

Dalakas, MC. Pharmacokinetics and bioavailability of corticosteroids in the treatment of neuromuscular diseases of the elderly. In: Cutler, NR, Narang, PK, eds. Methodological Concerns for Clinical Trials in Geriatrics, New York, Plenum Press, 283-298, 1986.

Dalakas, MC. Recent research issues in post-poliomyelitis muscular atrophy (PPMA): pathogenesis and rate of progression. In: Laurie, G., Raymond J. eds. Proceedings of 3rd International Post-polio Conference, Gazette International Networking Institute, St. Louis, MO, 39-40, 1986.

Dalakas, MC. Subacute painful myopathy from chronic propoxyphene napsylate (Darvon N) abuse. J. Am. Med. Assoc. 255:1709, 1986.

Dalakas, MC. The role of thymosin α_4 and interdigitating cells (IDC) in the thymic involvement of myasthenia gravis. Ann. NY Acad. Sci. (in press) 1987.

Dalakas, MC, Aksamit, AJ, Madden, DL, Sever, JL. Recombinant α_2 interferon in a pilot trial of patients with ALS. Arch Neurol. 43:933-935, 1986.

Dalakas, MC, Cunningham, G. Characterization of amyloid deposits in biopsies of 15 patients with "sporadic" (nonfamilial or plasma cell dyscrasia) amyloid polyneuropathy. Acta Neuropath. 69:66-72, 1986.

Dalakas, MC, Elder, G, Hallett, M, Ravitz, J, Baker, M, Papadopoulos, N, Albrecht, P. and Sever, JL. A long term follow-up study of patients with post-poliomyelitis neuromuscular symptoms. N. Engl. J. Med. 314:959-63, 1986.

Dalakas, MC, Elder, G, Hallett, M, Sever, JL. Post-poliomyelitis neuromuscular symptoms (A reply). N. Engl. J. Med. 315:897-898, 1986.

Dalakas, MC, London, WT, Gravell, M., Sever, JL. Polymyositis in an immunodeficiency disease in monkeys induced by a type D retrovirus. Neurology, 36:569-572, 1986.

Dalakas, MC, Pezeshkpour, GH. Neuromuscular complications of AIDS. Muscle and Nerve, 9:92, 1986.

Dalakas, MC, Pezeshkpour, GH, Gravell, M, Sever, JL. Polymyositis in patients with AIDS. J. Am. Med. Assoc. 256:2381-2383, 1986.

Dalakas, MC, Trapp, BT. Thymosin α_4 is a shared antigen between lymphoid cells and oligodendrocytes of normal human brain. Ann. Neurol. 19:349-355, 1986.

Elder, G, Dalakas, MC, Pezeshkpour, G, Sever, JL. Ataxic neuropathy due to ganglioneuritis after probable acute human immunodeficiency virus infection. Lancet (2): 1275-1276, 1986.

Ilyas, AA, Dalakas, MC, Brady, RO, Quarles, RH. Sulfated glucuronyl glycolipids reacting with antimyelin-associated glycoprotein monoclonal antibodies including IgM paraproteins in neuropathy: species distribution and partial characterization of epitopes. Brain Res. 385:1-9, 1986

O'Shannessy, JD, Ilyas, AA, Dalakas, MC, Mendell, JR, Quarles, RH. Specificity of human IgM monoclonal antibodies from patients with peripheral neuropathy. J. Neuroimmunol. 11:131-136, 1986.

Neuropathology, David A. Katz, M.D.

Diagnostic Neuropathology Service for NINCDS, and for all other Institutes, are provided by Dr. Katz. The Neuropathology Service is integrated with the Autopsy, Surgical Pathology, and Ultrastructural Pathology Sections and residency training program of the Laboratory of Pathology, NCI; a high priority is given to resident teaching. The brain is examined in approximately 100 of the 150 autopsies performed at NIH each year; fully 25% of these manifest significant primary or secondary neurological disease (autopsy material includes: malignant gliomas, cerebral metastases, dementias, AIDS). Brinecutting, held weekly, is scheduled so as to encourage participation by interested physicians and nurses. Relevant neuropathological findings are also presented at gross autopsy conferences. Selected cases are further utilized for neurological clinical conferences. Neurosurgical specimens include both in-house and submitted materials, for an annual total of approximately 175 cases; intra-operative frozen-section consultations are required in approximately 35 in-house cases per year (surgical material includes: malignant gliomas, AIDS, pituitary tumors, muscle biopsies).

The Neuropathology Service also functions in a collaborative manner to provide subspecialty expertise in a range of clinicopathologic investigations. Current active collaborations include: (1) participation in Alzheimer's disease protocols from both NIMH (Trey Sunderland, M.D.) and NIA (Stanley Rapoport, M.D.); (2) study of patients with progressive multifocal leukoencephalopathy (PML) (Sidney Houff, M.D., Ph.D.); (3) correlation of pathological and neuroimaging data in temporal lobectomy specimens from epileptic patients (William Theodore, M.D.); and (4) participation in experimental treatment protocols for malignant gliomas with both Surgical Neurology Branch, NINCDS, and Radiation Oncology Branch, NCI. Also, formal guidelines for the handling of known or suspected cases of Creutzfeldt-Jakob disease have been formulated and approved as Clinical Center policy (with David Henderson, M.D.).

Audiology, Anita Pikus, M.A. Audiology Unit, Chief

Clinical Activities:

The Audiology Clinic has undergone several changes since joining the NINCDS in July of 1986. After being closed for nearly six weeks because of renovations, we reopened newly configured with two new staff members, Dr. Daniel Sklare and Mrs. Mary Ann Mastroianni, a new audiology secretary. The change to full time for the Chief, Dr. Sklare and a secretary, promise to enhance our patient care and clinical research capabilities and to expand our participation in an effort to meet the requests for service.

The Audiology Program maintains close ties with many Institutes and is currently seeing large blocks of patients from NINCDS, NICHD, NIMH, and NCI. The following analysis demonstrates this year's breakdown by Institutes:

	<u>Audiologic Assessment</u>	<u>Auditory Evoked Potentials</u>
NINCDS	162	117
NIMH	52	50
NICHD	164	116
NIA	51	79
NCI	130	7
NHLBI	45	1
NIDDK	8	1
NIAID	42	5
NEI	26	13
NIAMS	3	0
TOTALS	683	391

Participation in Clinical Research:

The Audiology Clinic works in collaboration with many Branches within most of the Institutes, including the Inter-Institute Genetics Program. This past year has seen continuance of several ongoing studies as well as some important new studies. Alzheimer patients, especially from NIMH and NIA, continue to be sent to Audiology for both baseline and follow-up evaluations and we are just beginning a new collaborative effort with NIA on P300's in Alzheimer's disease. Other new collaborations have been developed with NICHD in the precocious puberty studies and with studies of glucocorticosteroid antagonist agent, RU486, on normal subjects. In addition, NIMH has begun a joint effort with Audiology Clinic in the study of the audiometry and vestibular function in panic state disorders. As part of that study, we look forward to establishing our vestibular laboratory. The Audiology Clinic continues to serve as a training environment for guest workers in otolaryngology and graduate students in audiology. The chief continues to serve as subject matter expert in audiology for the Office of Personnel Management (OPM).

Publications:

Sklare, D. A.: Auditory brainstem response laboratory norms: When is the data base sufficient? Ear and Hearing 8 (1): 56-57, 1987.

Sklare, D. A. and Denenberg, L. (1987). Interaural attenuation for TubephoneTM insert earphones. Ear and Hearing 1987, (in press).

Grimes, A. M., Grady C., and Pikus, A. Auditory Evoked Potentials in Patients with Dementia of the Alzheimer Type. Ear and Hearing 8(3): 57-161, 1987.

Paraprofessional Support Services

Linda Nee, MSW, is assigned to the Clinical Neuropharmacology Section, Medical Neurology Branch. She has been pursuing clinical and family studies, organizing field clinics and undertaking genetic counselling.

Helen Krebs, RN, is assigned to the Neuroimmunology Branch, where she is taking a major role in running a clinical trial of the use of cyclosporin in multiple sclerosis.

Marjorie Gillespie, RN, is assigned to the Experimental Therapeutics Branch, where she supports several aspects of the clinical program.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT				PROJECT NUMBER Z01 NS 02675-03 ODIR		
PERIOD COVERED October 1, 1986 through September 30, 1987						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evaluation of Neuromuscular Diseases						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)						
P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	DIR	NINCDS
Others:	Jacob Meer, M.D.	Acting Chief, EMG Lab.	OCD	ODIR	DIR	NINCDS
	Marinos Dalakas, M.D.	Medical Staff Fellow	OCD	ODIR	DIR	NINCDS
	Bruce Geller, M.D.	Medical Staff Fellow	OCD	ODIR	DIR	NINCDS
	Richard Dubinsky, M.D.	Medical Staff Fellow	HMCS	MNB	DIR	NINCDS
	Randall King, M.D.	Guest Worker	OCD	ODIR	DIR	NINCDS
COOPERATING UNITS (if any) None						
LAB/BRANCH Office of the Clinical Director, Office of the Director, Division of Intramural Research						
SECTION EMG Laboratory						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892						
TOTAL MAN-YEARS: 0.5		PROFESSIONAL: 0.4		OTHER: 0.1		
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Understanding of <u>neuromuscular disease</u> is founded on careful clinical observation, <u>electrodiagnostic studies</u> and <u>pathology</u> . This protocol has been carried out to learn more about established diseases, to characterize new diseases, to assess current methodologies and technologies and to refine old methods and develop new ones. We have started a study where we try to selectively record from the IA afferent fibers by means of a near nerve recording and mechanical tapping of the muscle. Several normal volunteers were studied and we plan to study more, as well as patients with relevant disorders. We have also studied and tried to understand a few patients with unusual disorders, such as familial hypoparathyroidism and myopathy, stiff-man syndrome, chronic fatigue syndrome, hereditary sensory motor neuropathy, hereditary liability to pressure palsies, and more.						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 N5 02743-01 ODIR																														
PERIOD COVERED October 1, 1986 through September 30, 1987																																
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Hearing Assessment in Normal Volunteers																																
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">P.I.:</td> <td style="width: 30%;">Anita Pikus, M.A., Chief</td> <td style="width: 15%;">Clinical Audiology</td> <td style="width: 10%;">OCD</td> <td style="width: 10%;">DIR</td> <td style="width: 5%;">NINCDS</td> </tr> <tr> <td colspan="6"> </td> </tr> <tr> <td>OTHERS:</td> <td>Daniel Sklare, Ph.D.</td> <td>Clinical Audiology</td> <td>OCD</td> <td>DIR</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Roswell Eldridge, M.D.</td> <td>Neurogenetics</td> <td>NEB</td> <td>DIR</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Mary Ann Mastroianni, M.S.</td> <td>Clinical Audiology</td> <td>OCD</td> <td>DIR</td> <td>NINCDS</td> </tr> </table>			P.I.:	Anita Pikus, M.A., Chief	Clinical Audiology	OCD	DIR	NINCDS							OTHERS:	Daniel Sklare, Ph.D.	Clinical Audiology	OCD	DIR	NINCDS		Roswell Eldridge, M.D.	Neurogenetics	NEB	DIR	NINCDS		Mary Ann Mastroianni, M.S.	Clinical Audiology	OCD	DIR	NINCDS
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This Fiscal Year the three year review was accomplished and subsequent changes in professional staff. Approval was given for continuance of the study in its revised form in early January 1987. Since the last review, approximately 75 normal volunteers have been studied, thereby providing the necessary data base for normal values in auditory evoked potentials using circumaural headphones. Norms were developed for different stimulus rates, intensities and polarities as well as for different age and sex groups. With the addition of new insert earphones and multi-frequency immittance equipment, we are looking forward to gathering normal values for these instruments and techniques as they will provide the necessary basis for comparison with the different age and disease populations studied in the Audiology Clinic as part of broader collaborative efforts. During the few months in which the revised protocol has been in place, we have seen 6 normal subjects for the insert earphone study of brainstem and later auditory evoked potentials. Software modifications on our multi-frequency immittance equipment seem now to be current and should allow us to begin gathering norms (as of July 1987). Naturally, as the Audiology Clinic is officially a service organization, our patients' needs come first and we see normal volunteers for this study only when no patient would be deprived on an audiologic assessment. However, age and sex matched normal controls are seen regularly as part of other collaborative protocols.																																

ANNUAL REPORT
October 1, 1986 through September 30, 1987

Neuroimaging Section, OD, DIR
National Institute of Neurological and Communicative Disorders
and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987
Neuroimaging Section, ODIR, DIR
National Institute of Neurological and Communicative
Disorders and Stroke

Giovanni Di Chiro, M.D., Chief

SUMMARY

Following is a summary of the major findings for the research protocols of the Neuroimaging Section in the fiscal year October 1, 1986 through September 30, 1987.

Radiographic and Radioisotopic Angiography of the Spinal Cord.
(Project #Z01 NS 01195-23) Angiographic studies of arteriovenous malformations and vascular tumors of the spinal cord have continued. Recent observations indicate that magnetic resonance imaging (MRI) is of higher value than previously estimated in the diagnostic assessment of these lesions (see following protocol). Also, on the basis of two observations, it has been possible to confirm that a myelopathic syndrome may originate from an intracranial dural arteriovenous malformation draining into spinal veins.

Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR). (Project #Z01 NS 02073-14) CT studies of conditions such as degenerative diseases of the CNS, cavities of the brain stem and spinal cord, and brain and spinal cord tumors and malformations have continued.

The NMR imaging research has developed along several lines:

- 1) Studies of pulsatile CSF flow using longitudinal imaging (flow direction in the image plane) and phase reconstruction. This new method is advantageous for its speed and simplicity; pulsatile flow direction and velocity may be easily assessed, and there is some indication that the method will give information about CSF bulk flow.
- 2) Studies of artifactual linear regions of abnormal signal intensity along the length of the spinal cord. These artifacts, which are related to sampling (Gibbs phenomenon), have been confused with cord pathology (syringomyelia, arteriovenous malformations).
- 3) Continuing the study of patients with spinal cord pathology, particularly arteriovenous malformations (AVM). The MRI diagnostic yield with these lesions is continuously improving; of particular interest is the recently acquired capability to recognize the disappearance or reduction in size of AVMs after surgical or embolization treatment. This obviates the need of invasive arteriographic controls.

- 4) Continuing in vivo and in vitro investigation of the relaxation times (T_1, T_2) of extravasated intracranial blood. Changes in T_1 and T_2 are, in great part, related to the chemical modifications and progressive denaturation of hemoglobin.
- 5) Trying to learn more about the NMR signal of various abnormal tissues. Particular attention has been devoted to the signal from CNS tumors of various types and grades. We are also engaged in a comparative "in vivo/in vitro" study of T_1 and T_2 of normal and pathological CNS tissues.
- 6) Comparing clinical MRI imaging results with those of CT and particularly PET in a variety of abnormal conditions, starting with CNS tumors.
- 7) Successful initial imaging with an experimental, small bore (animal studies), 2 Tesla imaging-spectroscopy NMR device. This is the first necessary step in the development of our imaging plus spectroscopy program.

Positron Emission Tomography (Project #Z01 02315-10) The FDG-PET method of tumor evaluation has been extended from the gliomas to other intracranial tumors, particularly meningiomas. We have clear indications that FDG-PET is an excellent method to predict "post-removal" recurrence of meningiomas.

Confirmation has been obtained that the FDG-PET method is the optimal procedure for differentiating tumor recurrence from postradiation and/or postchemotherapy cerebral necrosis.

An analysis of the cortical glucose metabolism in the hemianopsias, with emphasis on the homonymous field defects, has been completed. In cases of hemianopsia, the appropriate primary and associative visual cortices show marked hypometabolism.

A "split" or "test-retest" FDG-PET method has been developed. This method permits us to assess "baseline" regional (or global) glucose utilization rates of the brain with "post-stimulation" (motor-sensory, drug-induced) values.

A study of the correlation between glucose utilization ratio and brain size has been completed. On the basis of PET examinations carried out with three different scanners the cerebral metabolic rate per unit volume has been found to be inversely proportional to brain size.

Finally, PET studies of ^{18}F labeled L-Dopa in monkeys have been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01195-23 ODIR									
PERIOD COVERED October 1, 1986 through September 30, 1987											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Radiographic and Radioisotopic Angiography of the Spinal Cord											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Giovanni Di Chiro, M.D., Chief, Neuroimaging Section, ODIR, NINCDS											
OTHERS: <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">J. L. Doppman, M.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 30%;">DRD, CC</td> </tr> <tr> <td>E. H. Oldfield, M.D.</td> <td>Acting Chief</td> <td>SN, NINCDS</td> </tr> <tr> <td>S. M. Larson, M.D.</td> <td>Chief</td> <td>SN, CC</td> </tr> </table>			J. L. Doppman, M.D.	Chief	DRD, CC	E. H. Oldfield, M.D.	Acting Chief	SN, NINCDS	S. M. Larson, M.D.	Chief	SN, CC
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E. H. Oldfield, M.D.	Acting Chief	SN, NINCDS									
S. M. Larson, M.D.	Chief	SN, CC									
COOPERATING UNITS (if any) Diagnostic Radiology, and Nuclear Medicine Departments, Clinical Center, NIH; Surgical Neurology Branch, NINCDS; Medical Examiner's Office, Department of Public Health, Philadelphia, PA											
LAB/BRANCH Office of the Director, DIR											
SECTION Neuroimaging Section											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892											
TOTAL MAN-YEARS 0.3	PROFESSIONAL: 0.3	OTHER:									
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Selective arteriography</u> (radiographic) of the spinal cord is a diagnostic technique which has proven to be informative in cases of arteriovenous malformation (AVM), tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord. <u>Radioisotope angiography</u> of the spinal cord offers some advantages as a screening method, and in certain types of intraspinal pathology may give information not available by any other diagnostic test. Experience with the techniques of <u>dynamic computed tomography</u>, <u>digital subtraction angiography</u> (DSA), <u>positron emission tomography</u> (PET) using <u>¹⁸F-2-deoxyglucose</u> and nuclear <u>magnetic resonance imaging</u> (MRI) of the spine indicates that these methods may be useful screening and follow-up procedures in the evaluation of certain vascular lesions and tumors of the spinal cord.											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO NS 02073-14 ODIR																		
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TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR)																				
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LAB/BRANCH Office of the Director, DIR																				
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Computed Tomography in its transmission (CT), emission (PET, SPECT), and Nuclear Magnetic Resonance (NMR) modalities, represents the main research area of the Neuroimaging Section. <u>CT</u>: Ongoing clinical and experimental research projects in transmission CT, include studies of tumoral, degenerative, demyelinating and atrophic processes of the brain, plus hydrocephalus, brain edema, postradiation necrosis, and surgically correctable lesions in young patients affected by chronic epilepsy. <u>NMR</u>: Our NMR imaging research is developing along seven main lines: 1) assessment of pulsatile CSF flow using longitudinal imaging; 2) recognition of artifactual linear regions of abnormal signal intensity along the length of the spinal cord; 3) study of patients with spinal cord pathology, particularly arteriovenous malformations (AVM); 4) continuing the <u>in vivo</u> and <u>in vitro</u> investigation of the relaxation times (T₁, T₂) of extravasated intracranial blood; 5) trying to learn more about the NMR signal of various abnormal tissues; 6) comparing clinical MRI imaging results with those of CT and particularly PET; 7) successful initial imaging with an experimental, small bore (animal studies), 2 Tesla imaging-spectroscopy device.																				

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TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Positron Emission Tomography																																
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Positron Emission Tomography (PET) using ^{18}F-2-deoxyglucose (FDG) allows us to obtain anatomical data (e.g., axial transverse or coronal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate, and measurement of storage, degradation and turnover of tagged metabolites). Besides FDG, other radiopharmaceuticals (tagged with ^{18}F, ^{15}O, ^{11}C, ^{13}N) can be used with PET to study the BBB, oxygen metabolism, protein synthesis, and neuroreceptors. The unique property of PET is that it provides <u>physiologic and pathophysiologic information</u> not available with any other imaging procedure. ----- * Continued: <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">I. J. Kopin, M.D.</td> <td style="width: 30%;">Director</td> <td style="width: 30%;">DIR, NINCDS</td> </tr> <tr> <td>C. C. Chiueh, Ph.D</td> <td>Pharmacologist</td> <td>CBI, LCM, NIMH</td> </tr> <tr> <td>R. M. Cohen, M.D.</td> <td>Chief</td> <td>CBI, LCM, NIMH</td> </tr> <tr> <td>W. H. Theodore, M.D.</td> <td>Neurologist</td> <td>MN, NINCDS</td> </tr> <tr> <td>E. H. Oldfield, M.D.</td> <td>Acting Chief</td> <td>SN, NINCDS</td> </tr> <tr> <td>D. C. Wright, M.D.</td> <td>Staff Physician</td> <td>SN, NINCDS</td> </tr> <tr> <td>C. V. Kufta, M.D.</td> <td>Staff Physician</td> <td>SN, NINCDS</td> </tr> <tr> <td>M. Hallett, M.D.</td> <td>Clinical Director</td> <td>DIR, NINCDS</td> </tr> <tr> <td>R. J. Polinsky, M.D.</td> <td>Staff Physician</td> <td>MN, NINCDS</td> </tr> <tr> <td>L. Sokoloff, M.D.</td> <td>Chief</td> <td>LCM, NIMH</td> </tr> </table> 21 - ODIR/DIR (NIS)			I. J. Kopin, M.D.	Director	DIR, NINCDS	C. C. Chiueh, Ph.D	Pharmacologist	CBI, LCM, NIMH	R. M. Cohen, M.D.	Chief	CBI, LCM, NIMH	W. H. Theodore, M.D.	Neurologist	MN, NINCDS	E. H. Oldfield, M.D.	Acting Chief	SN, NINCDS	D. C. Wright, M.D.	Staff Physician	SN, NINCDS	C. V. Kufta, M.D.	Staff Physician	SN, NINCDS	M. Hallett, M.D.	Clinical Director	DIR, NINCDS	R. J. Polinsky, M.D.	Staff Physician	MN, NINCDS	L. Sokoloff, M.D.	Chief	LCM, NIMH
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Publications:

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3. Bairamian, D., Di Chiro, G., Theodore, W.H.: Epilepsy and cerebellar hypometabolism. Ann Neurol. 20:649, 1986.
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12. Kattah, J.C., Cupps, T.R., Di Chiro, G., Manz, H.J.: The Potential Diagnostic Role of Magnetic Resonance Imaging in Central Nervous System Vasculitis. J Neurology. In Press.
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14. Doppman, J.L., Di Chiro, G., Dwyer, A.J., Frank, J.L., Oldfield, E.H.: Magnetic Resonance Imaging of Spinal Arteriovenous Malformations. J Neurosurg. In Press.
15. Di Chiro, G., Ericson, K.: Emission Tomography. Acta Radiol. In Press.
16. Di Chiro, G.: Positron Emission Tomography Using [¹⁸F] Fluorodeoxyglucose in Brain Tumors: A Powerful Diagnostic and Prognostic Tool. Investigative Radiology. In Press.
17. Blacklock, J.B., Oldfield, E.H., Di Chiro, G., Tran, D., Theodore, W., Wright, D.C., Larson, S.M.: Selective Suppression of Glucose Utilization in Normal Brain Versus Gliomas: Positron Emission Tomography During Barbiturate Coma in Humans. J Neurosurg. In Press.
18. Brooks, R.A., Hatazawa, J., Di Chiro, G., Larson, S.M., Fishbein, D.: Human Cerebral Glucose Metabolism Determined by Positron Emission Tomography: A Revisit. J Cereb Blood Flow Metabol. In Press.
19. Fishbein, D.S., Chrousos, G.A., Di Chiro, G., Wayner, R.E., Patronas, N.J., Larson, S.M.: Glucose Utilization of Visual Cortex Following Extra-occipital Interruptions of the Visual Pathways By Tumor: A Positron Emission Tomography Study. J Clin Neuro-Ophthalmol. In Press.
20. Brown, R.T., Polinsky, R.J., Di Chiro, G., Pastakia, B., Wener, L., Simmons, J.R.: Differentiation Between MSA and IOH by MRI. J Neurol Neurosurg Psychiatry. In Press.

21. Hatazawa, J., Brooks, R.A., Di Chiro, G., Campbell, G.: Global Cerebral Utilization is Independent of Brain Size: A PET Analysis. J Comput Assist Tomogr. In Press.
22. Dalakas, M.C., Hatazawa, J., Brooks, R.A., Di Chiro, G.: Lowered Cerebral Glucose Utilization in Amyotrophic Lateral Sclerosis. Ann Neurol. In Press.
23. Di Chiro, G., Hatazawa, J., Katz, D., Rizzoli, H.V., De Michele, D.J.: Glucose Utilization by Intracranial Meningiomas as an Index of Emission Tomography Study. Radiology. In Press.

ANNUAL REPORT

October 1, 1986 - September 30, 1987

Instrumentation and Computer Section

National Institute of Neurological and Communicative Disorders and Stroke

Table of Contents

ORGANIZATIONAL STRUCTURE AND SERVICES

INSTRUMENTATION

COMPUTER SUPPORT

DISTRIBUTION OF ENGINEERING, COMPUTER, AND FABRICATION SERVICES

INSTRUMENTATION & COMPUTER SECTION

National Institute of Neurological and Communicative Disorders & Stroke

October 1, 1986 - September 30, 1987

Bruce M. Smith, Ph.D., Chief

The Instrumentation and Computer Section (ICS) provides technical support for investigators of NIMH and NINCDS Divisions of Intramural Research (DIRs) by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing; and (4) managing a central computer facility consisting of a multiuser VAX-11/750, two PDP-11 image processing systems, and a network of Macintosh personal computers and LaserWriter printers.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electromagnetic interference in data measurement systems, and equipment and computer purchases. Formal or informal instruction for individual investigators or groups are offered by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS will take on the project. If a commercial product will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Chief agrees to accept a project, the investigator submits a standard ICS work request form, signed by his Lab Chief. This form states the nature of the instrument or service requested, and should contain as many details and specifications as the investigator can provide. The project is then assigned to an engineer or computer staff member, who confers with the investigator to formulate a complete set of specifications and a cost estimate for the project. ICS does not charge for services, but upon completion of the project the investigator's laboratory or branch is billed for the cost of the components used. Reimbursement of funds takes place at the beginning of the next fiscal year.

INSTRUMENTATION

The Section has a staff of five engineers, four computer specialists and four technicians to design and produce special-purpose instrumentation. The recent availability of powerful, low-cost personal computers and single-chip microprocessors has broadened the Section's approach to instrumentation development. It is often appropriate for an engineer, a technician, and a computer specialist to work together to combine unique electronic or mechanical hardware, a personal computer or microprocessor, and applications software to produce a flexible, cost-effective solution to complex instrumentation problems. The following are brief descriptions of the Section's major projects, taken from a total of 285 projects undertaken this year.

PATIENT MONITORING

Ambulatory Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the hardware and software which forms the system.

Monitor. The current version of the PAM has a memory capacity of 1024 locations and is in its fifth year of production. Fabrication, testing, and calibration of a set of 50 units begun last year has been completed and another set of 50 monitors is now in the initial phase of fabrication. Approximately 135 monitors are in use, with the Section providing battery changes and repairs as needed. Further development of the injection-molded plastic case for the PAM is necessary to improve its ability to reject interference from static electricity. Cases molded with an ABS plastic containing minute stainless steel fibers did not provide adequate protection during the dry winter months.

Computer Support. The Section has developed a readout system based on the Macintosh personal computer coupled to a microprocessor-controlled PAM interface. A comprehensive PAM program has been written for the Macintosh to handle data readout and disk filing, graphical data editing, construction of continuous data files and raster plots, and formation of tabular data sets for transfer into spreadsheet and statistical applications. Recent additions to the program include a provision to expedite transfer of activity data to DCRT's IBM mainframe for analysis using SAS, support for multiple-page raster plots, and support for faster sampling rates. A related program has been developed on the VAX to convert PDP-11 PAM files into Macintosh format. Eight of the PAM interfaces have been fabricated for DIR studies; an additional five units have been supplied in support of collaborative efforts outside of the DIRs.

Multi-bed Unit. The Section is providing technical assistance for specifying and implementing equipment for a new, four bed patient monitoring unit in the Biological Psychiatry Branch (NIMH). The equipment should be flexible enough to accommodate many different protocols and will include the following: (1) a system for monitoring and recording patient physiological data, including blood pressure, EEG, temperature, respiration and pulse rate; (2) video monitoring and recording of each subject multiplexed with selectable physiologic parameters; (3) patient self-rating equipment and recording; and (4) topographic EEG brain mapping. Several computer systems will need to be integrated and software developed to control the experiments, log the data, and provide off-line analyses of the results.

ANIMAL MONITORING

Microprocessor-based Rotometer. A third generation animal rotation monitor incorporates an 8-bit microprocessor to determine the clockwise and counterclockwise rotations of 16 rodents in cylindrical cages and to hold this data for input into the serial port of a Macintosh computer. Software has been written which uses the capabilities of the Macintosh to store the data on disk, and to display the data in real-time histogram form. Powerful statistical programs now available allow complete data analysis directly on the Macintosh. The combination of the Macintosh and the high-speed microprocessor system reduces the number of integrated circuits in the design by a factor of 20 over previous ICS 16-channel designs. Two 16-channel units have been fabricated.

Microprocessor-based Wheel Rotometer. A 32-channel system for monitoring the running-wheel rotations of rodents is being developed by utilizing the Macintosh-microprocessor combination described above for the 16-channel rotometer. In this case, a cam attached to the wheel axle activates a microswitch to sense the rotations. The microprocessor debounces the switch inputs, calculates complete rotations and pulses the corresponding front panel indicators, and transmits to the Macintosh at programmed intervals the accumulated rotations for each channel. The Macintosh program will store the data and provide real-time histogram displays.

Event Timer and Recorder. A program called Timer has been developed for observing, recording, and timing behavioral events using a Macintosh computer and a custom keypad. Keys on the keypad can function as pushbuttons for short events, toggle switches for long events, or keypad locks. While timing, the program saves each event that occurs and updates a display of the keypad status and the cumulative frequency and total elapsed time for each event. The sequence of events acquired in a session is saved to disk for off-line analysis by a separate program. The user can start and stop the session clock with the keypad or can optionally schedule the program to terminate automatically after a specified elapsed time.

IMAGE PROCESSING

Quantitative Morphometrics System. ICS has developed a program called MacMeasure for counting and measuring structures in photographs by use of a Macintosh computer and a digitizing tablet. The length, perimeter and area of structures can be easily measured, sequentially numbered, scaled, mapped and stored on disk. The scale and unit of measurement can be specified. Results can be listed on the screen, printed, or saved to disk for later analysis by other programs.

Microscope-Plotter Interface. An interface has been developed that allows the movements of an X-Y plotter to precisely follow the X-Y movements of a microscope stage. The outputs from linear potentiometers attached to the microscope stage are connected to amplifiers which provide proportional voltages to the inputs of the plotter. The pen of the plotter is therefore coupled to the movements of the microscope stage making it possible to draw or count the image at a magnification which is independent of that used for observation. The circuitry provides a wide range of gain and offset control so that a small image area may be plotted over the full page.

LaserWriter-Matrox Plotting Program. A program called LMplot was created to allow users of several off-line neurophysiological analysis programs to easily plot many Matrox plot files on any PostScript printer. The program allows the user to specify a range of input files for plotting, to selectively print from one to 24 plots on a single page, and to invert a plot file before printing (a feature useful for printing plot files created by earlier analysis programs). LMplot prompts the user for all input, offers reasonable defaults, and checks user input before proceeding. The program was created in OMSI Pascal-1 for PDP-11 computers running RT-11 and with a few, documented exceptions, adheres to the ANSI Pascal standard.

Microscope-based Video Imaging Systems. ICS is furnishing considerable software support to two laboratories developing real-time image processing systems for the study of the use of voltage sensitive dyes to measure membrane potential changes, cell and synaptic morphology using enhanced optical microscopy, and dendrite morphology using fractal analysis.

ELECTROMECHANICAL CONTROLLERS

Hex Valve Controller. A digital controller was developed to operate six flow valves for fluid infusion in cell studies. Latching valves with low power dissipation were used so that the fluid temperature would not be affected by the valve temperature. Each valve may be controlled manually or by a digital input signal; switching between the two modes can be done only when that valve is closed. A single pushbutton closes all valves and resets the controller to manual control; the AC line voltage is also monitored to provide a "fail-safe" shutdown of all valves.

Filter Selector. A stepper motor controller was designed to quickly and accurately alternate two UV filters in and out of the excitation light path of a Zeiss microscope-computer system used for cell fluorescence studies. A special filter holder and mechanical base were designed to interface with the microscope. The controller sets the speed and degrees of movement of the filter holder for a

particular experimental application; controller operation may be triggered manually or periodically by the system computer.

STIMULATORS AND SIGNAL PROCESSORS

Kindling Stimulator. Seizure activity can be triggered in rats by injecting a series of current pulses directly into the brain via microelectrodes in a process known as kindling. Two precision digital instruments were constructed to generate unipolar or bipolar pulse trains, with current ranges of 2 to 2000 μ A. The pulse train can be set to contain 1 to 999 pulses with interpulse intervals of 10 to 990 msec.; pulse widths are fixed at one msec. in duration. The isolated constant current source output is sampled and displayed on a calibrated panel meter.

Electrochemical Detector. In liquid chromatography, amperometric detectors are used to monitor oxidation or reduction currents to determine a compound's identity. In this type of measurement, a precision voltage, unique for a specific substance, is applied to a small cell of liquid and the resultant current (10-100 nA) is measured. A simple instrument was developed to supply the precision voltage and to capture the resultant current signal. Background current is removed by an offset adjustment and the detected signal amplified by a variable low-pass filter for collection on a strip chart recorder. Five dual-channel instruments were fabricated this year.

Wavelength Ratiometer. Analog circuitry has been developed to produce a voltage proportional to the ratio of light at two wavelengths (480Å and 405Å) after absorption by a tissue sample. The absorbed intensity at each wavelength is first detected by a photodiode-current-to-voltage converter circuit. A ganged, matched set of multipliers allows the magnitude of these voltages to then be manually scaled by exactly the same factor. The resultant voltage levels become the numerator and denominator in a precision analog divider. The ratio, numerator, and denominator are all buffered for external data acquisition, and can be displayed on a panel meter.

COMPUTER SUPPORT

In addition to the development of special instrumentation systems, the ICS provides support for individual laboratory computer systems and maintains central computer facilities for high-capacity data storage, complex off-line data analysis, image processing, scientific word processing, and high-quality printing and plotting. These support services are detailed below.

LABORATORY COMPUTERS

Small minicomputers, and more recently, personal computers, are widely used in the DIR laboratories for real-time data acquisition and control. ICS provides consultation on the specification and selection of these systems and helps the scientist in the procurement, installation and maintenance of the equipment. Training in operating systems, programming languages and maintenance issues is available for scientists or laboratory support personnel. Manpower limitations make it difficult for ICS to provide complete programming for specific individual applications. ICS personnel are always available for consultation and will aid the investigator in writing the difficult time and data dependent sections of real-time programs. Section programming efforts in support of laboratory applications are concentrated on developing and maintaining a library of routines which are specifically designed to be incorporated into investigators' programs. ICS personnel also evaluate commercial software or applications programs from other research facilities to determine their utility for DIR laboratory systems.

For more than a decade ICS has developed and supported laboratory computer systems based on DEC PDP-11 minicomputers, the RT-11 operating system and selected programming languages. This standardization of equipment and software techniques has been very efficient and allowed the Section's small computer staff to support more than 60 of these individual systems.

In spite of this success and the Section's considerable accumulated knowledge and experience, the 16-bit PDP-11 with its limited memory capacity is often no longer the best choice for laboratory applications. Advanced 32-bit personal computers now offer more powerful and cost-effective solutions. ICS has selected the Apple Macintosh family of computer systems as our new standard for support of scientific applications. The Section has already developed considerable experience using the Macintosh Plus to provide innovative solutions for low-speed laboratory data acquisition projects. For the acquisition of real-time data and control of laboratory devices at high speeds, the Section is now beginning development of utility routines and applications software for the new Macintosh II. The Macintosh II has an advanced open architecture, built-in high resolution monochrome or color graphics, and a high-speed 32-bit main processor with a math co-processor. These features will provide several times the performance of the PDP-11 at a significant reduction in cost.

VAX FACILITY

ICS manages a DEC VAX-11/750 computer system that is available to all DIR investigators. The VAX is located in Building 36, in space furnished by the Laboratory of Cerebral Metabolism, NIMH. VAX/VMS is the primary operating system, though a System V UNIX shell with Berkeley extensions is also available. Over 50 users in Building 36 and Building 10 have hard-wired cable connections or leased lines for high-speed communication with the VAX. Users can also gain access on a dial-up basis at 1200 or 2400 baud.

The Section supports software and sequence databases on the VAX for the entry, analysis, and publication of nucleic acid and protein sequences. The most popular sequence analysis software comes from the University of Wisconsin Genetics Computing Group and includes about 40 programs, a 300 page illustrated manual, and complete on-line help. ICS also provides the complete GenBank nucleic acid database and the NBRF protein database with quarterly updates.

DataPlot, an interactive program for curve fitting and graphics, is also available on the VAX. Similar to MLAB, DataPlot is a tool for experimenting with mathematical models, as well as summarizing and analyzing data. Publication-quality graphs produced by DataPlot can be plotted on a Talaris laser printer using utilities created with the Section's PlotLib graphics library.

IMAGE PROCESSING FACILITY

ICS maintains a central facility in Building 36 for image processing consisting of two PDP-11 computers with image array processors, a rotating drum scanner, and a TV camera digitizer. These systems are used for numerous applications, including evaluation and quantification of CAT or ECAT images, densitometric analysis of autoradiographs, and analysis of two-dimensional electrophoresis gels. A camera station is available to produce color hardcopy using Polaroid SX-70 or 35 mm film. The Section is also beginning development of a Macintosh II image processing system which will have most of the capabilities of the PDP-11 based systems but will be smaller, more reliable, less expensive, and easier to use.

PERSONAL COMPUTER FACILITY

Included in the Section's central facility in Building 36 are two Macintosh Plus computers, a modem, a LaserWriter Plus printer, and a variety of software that DIR scientists can use for statistical analysis, for communicating with DCRT's mainframes and MEDLINE , and for word processing, including creation of posters, slides, and publication-quality charts and graphs. Both Macintoshes are also connected to the Section's VAX and can be used to emulate VT-100 or Tektronix 4014 terminals. ICS plans to respond to the heavy use of these facilities by providing additional Macintosh computers in the near future.

While the main emphasis in personal computers by ICS staff has been on the Macintosh system, a smaller effort is under way to develop expertise with the IBM personal computer. One staff member has become involved in the DCRT Personal Workstation Office lead user program and continues to gain expertise through advanced DCRT courses. ICS is developing an IBM-AT PC system as an engineering workstation and as a prototype for laboratory data acquisition projects.

ENGINEERING, COMPUTER, AND FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories and branches. We have listed only the major users.

<u>LABORATORY OR BRANCH</u>	<u>PERCENT</u>
Medical Neurology, NINCDS	17.78
Neurophysiology, NINCDS	11.82
Biological Psychiatry, NIMH	7.21
Cerebral Metabolism, NIMH	6.18
Child Psychiatry, NIMH	5.67
Neurophysiology, NIMH	4.93
Clinical Science, NIMH	4.79
Clinical Psychobiology, NIMH	4.41
Neuropsychology, NIMH	4.02
Experimental Therapeutics, NINCDS	3.01
Molecular Biology, NIMH	2.87
Cell Biology, NIMH	2.54
Surgical Neurology, NINCDS	2.52
Biophysics, NINCDS	2.48
Neural Control, NINCDS	1.98
Neuropsychiatry, NIMH	1.79
Molecular Genetics, NINCDS	1.67
Clinical Neuroscience, NIMH	1.19
Neurochemistry, NINCDS	0.95
Neurobiology, NINCDS	0.81
Molecular Biology, NINCDS	0.78
Neurochemistry, NIMH	0.70
Central Nervous System Studies, NINCDS	0.65
Neuropathology & Neuroanatomical Sciences, NINCDS	0.44
Neuro-otolaryngology, NINCDS	0.32
Experimental Neuropathology, NINCDS	0.20
*NIMH (TOTAL)	46.64
*NINCDS (TOTAL)	45.47
*NICHHD (TOTAL)**	6.44
*NIAAA (TOTAL)***	<u>1.45</u>
	100.00

*These figures represent our total effort; they include labs not listed individually.

**This figure represents our support of NICHHD which loans the Section 1/2 FTE.

***This figure represents our support of collaborative efforts between NIAAA and NIMH.

ANNUAL REPORT

ANIMAL HEALTH AND CARE SECTION

NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISORDERS AND STROKE

October 1, 1986 through September 30, 1987

William T. London, D.V.M., Acting Chief

The Animal Health and Care Section (AHCS), as its name states, oversees the health needs and provides support in the care for animals used in the research efforts of investigation in the DIR, NINCDS. It fulfills these goals by: (1) ordering animals from licensed and reliable dealers; (2) housing and providing care for the animals in accordance with NIH guidelines; and (3) observing the animals and providing health care to those in need.

The AHCS also serves in an advisory capacity by informing investigators on current guidelines for humane care and use of animals and on such matters as proper anaesthetics, best species or strain of animal for particular studies, appropriate surgical procedures, etc.

The Chief, AHCS, serves as a member of the Animal Care and Use Committee (ACUC) of the NINCDS. In this capacity, he utilizes his expertise to advise and guide the ACUC in establishing and implementing the Institute's goals for animal care and use. The Chief, AHCS, screens research protocols for appropriate animal care and use practices prior to the review by the ACUC. After the research protocols are approved by the ACUC, the AHCS files and monitors the protocols for compliance, uses ordering policies established by the AHC Section, and insures that all protocols have been reviewed and that sufficient space and proper caging are available prior to placing orders for the animals.

The Chief, AHCS, also organizes and coordinates the biannual review of the NINCDS animal facilities conducted by the ACUC.

Within the AHCS, the animal care technicians are organized as a single service entity under the supervision of the Chief, AHCS.

Two major tasks were undertaken by the Section this FY:

1. Preparation of an NINCDS assurance statement for its animal facilities at NIH and a joint statement with NIAID for animal facilities of both Institutes located at the NCI, Frederick Cancer Research Facility. These were sent to the Scientific Director, DIR, NINCDS for approval and submission to ACUC, NIH.

2. Provide advice and direction for the development of animal facilities for the Institute. This included ascertaining that facilities and guidelines adhere to proper and humane care of all laboratory animals to meet the requirements of the American Association of Accreditation of Laboratory Animal Care (AAALAC).

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Biophysics
National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
October 1, 1986 thru September 30, 1987
National Institute of Neurological and Communicative
Disorders and Stroke
Laboratory of Biophysics
William J. Adelman, Jr., PhD, Chief

INTRODUCTION

Research in the Laboratory of Biophysics (LB) is concerned with achieving an understanding of the molecular basis for the functioning of neuronal cells, tissues and systems. The laboratory has had two units. The Woods Hole (WH), Massachusetts unit has been composed of two Sections: Neural Membranes (NM) and Neural Systems (NS), both located at the Marine Biological Laboratory. The Bethesda unit is the Section on Molecular Biophysics, located in Bldg. 36 at NIH. FY 1987 marks the twelfth and last year of continuous operation of the Woods Hole unit of LB.

LB studies membrane ion channels, and has developed concepts of channel structure and function which have provided an understanding of the mechanisms for generating nerve impulses, synaptic activity, and higher integrative behavior of nervous systems.

Biophysical methods have been coupled with modern ultrastructural and biochemical techniques in order to investigate complicated neuronal mechanisms at fundamental levels. LB's ultrastructural studies in Woods Hole cease with this report. Work will continue in Bethesda on membrane channels; the Section on Neural Membranes will move to Bethesda; and the Section on Neural Systems will be incorporated into a new Laboratory unit, the Laboratory of Cellular and Molecular Neurobiology.

Section on Neural Membranes.

The Section on Neural Membranes has used electrical, chemical, optical, electron optical, mathematical and computer science techniques to investigate the function of neural cells and tissues at limits approaching molecular levels. Molecular structures responsible for membrane ionic channel function and axoplasmic transport were sought. Model systems have been constructed, tested and developed to simulate a variety of neuronal functions.

The Section has completed the fourth, fifth and sixth phases of its study of the sodium channel gating mechanism in the squid axon. Gating current records were obtained from internally perfused squid giant axons with intact sodium inactivation in sinusoidally driven dynamic steady-states and as dynamic transients as functions of the mean membrane potential and the frequency of the command sinusoid. Records were obtained before and after long duration hyperpolarizing voltage conditioning. The non-linear analysis consisted of determining and interpreting the harmonic content in the current records. The results indicate the presence of a slow inactivation process. The dynamic steady-state data show that slow inactivation gating can be removed with internal protease treatment. Slow inactivation of the gating current has been shown to be related to the equivalent phenomenon in the macroscopic ionic currents.

The temperature dependence of the sodium channel gating current has been determined at several mean membrane potentials. Between 4°C and 14°C the Q_{10} s of the primary and secondary gating process are about 3. At temperatures above 14°C the Q_{10} s of these processes rise somewhat. Overall all values of Q_{10} lie between 2.5 and 5. These high Q_{10} values indicate that the measured gating current is generated by a high order chemical process, and is directly related to the ionic current conductance mechanism.

Further work on voltage dependence of the gating process has allowed for model refinement. These refinements are being incorporated into the model of sodium channel gating presented previously.

The project comparing ionic current channels in nerve and heart cell membranes has elucidated the role of potassium channel currents in spontaneous activity of excitable membranes. Of particular significance has been the finding that derivatives of triethylammonium ions block potassium channels according to their molecular size. Small blockers, such as methyltriethylammonium and tetraethylammonium block without altering channel gating; large blockers, such as Bn-pentyltriethylammonium and n-nonyltriethylammonium, alter channel gatings as well. Moreover, potency of blockade increases with size of the blocker. These results are relevant to the mechanism of antifibrillatory drug action in the heart. In particular, potassium channel blockade appears to be the critical factor in preventing fibrillation. For example, a potent blocker of potassium current, such as n-nonyltriethylammonium, produces a significant antifibrillation effect, whereas a relatively ineffective blocker of potassium current, such as methyltriethylammonium produces relatively little antifibrillatory effect. The mechanism of antifibrillatory action is related to current flow between cells in the heart. By impeding the flow of potassium ions, antifibrillatory compounds change conduction pathways in the heart. Instead of running across individual cell membranes, current now flows more readily between cells. The process synchronizes the beating of heart cells and stops fibrillation, as the cells again contract in unison. The squid axon has played a significant role in this work by providing a rapid and effective screening technique for testing the potency of potassium channel blockade of putative antifibrillatory compounds.

The effort by the Section in improving image processing techniques continues in both electron and light microscopy. Three-dimensional electron microscopy of thick sections using successive specimen tilts and Fourier processing of images was advanced by the design, manufacture, and installation of a new EM column screen for digital video visualization of thick sections. Making use of LB's computer based image processing system, both light and electron microscopic images have been analyzed.

Work on primary culture of squid neurons has been highly successful and these cultured neurons are now used routinely for biophysical and physiological studies by LB and others. These cells have been identified as neurons by immunofluorescent assay with tetanus toxin. Somal diameters range from 5 to 40 μ m and neurite processes are several mm long. Embryonic cells from two other molluscs, Hermisenda and Octopus, also grow under the same conditions. Neurite growth has been observed using the image processing system described above.

Squid cultured cells (and several mammalian cell lines) send out filopodia as they attach to the substrate. The slower growing squid cells facilitate the study of filopodia motility. A "slip-knot" action is present which has not been described previously. This manifestation of motility appears to be a new fundamental dynamic property of cells. These studies are now providing new insights on the development of neurons and on neuronal connectivity.

A key question we are addressing is how calcium influx into a secretory cell causes the secretion of neurotransmitters and hormones. Much of the work of the Section involves testing a hypothesis that we developed in an attempt to answer this basic question. According to this hypothesis, secretory vesicles contain both calcium-activated potassium channels and anion channels. When calcium enters the cell, it binds to sites on the calcium-activated potassium channels, allowing these channels to open. The opening of these channels and the presence of anion channels cause potassium ions and anions to enter the vesicles, thus increasing their osmotic pressure and causing an influx of water. For those vesicles situated very close to the cell plasma membrane, this could lead to fusion with the membrane and exocytosis of the vesicle contents. This hypothesis has been very useful not only in providing a possible answer to the basic question we are addressing, but also in leading to experiments that are of interest whether or not the hypothesis is true.

The description presented above applies to the general case, where an increase in internal calcium concentration causes an increase in the amount of secretion. An exception to this general rule occurs in the parathyroid cell, where an increase in internal calcium concentration causes a decrease in the amount of secretion. According to a reasonable extension of our hypothesis, an increase in secretion would still be correlated with the opening of calcium channels, but the channel opening would have a different calcium dependence. To fit the experimental data, the dose-response curve for channel opening must have a region where increased calcium reduces the probability of channel opening.

We have previously employed reconstitution experiments to test whether the channels required by the model are present in vesicles of neurosecretory cells. In these experiments, channels from vesicles of bovine neurohypophysis were reconstituted into lipid bilayers and their electrical properties were determined. Both calcium-activated potassium channels and anion channels were found to be in the vesicles, in agreement with the model. We have now determined the properties of the calcium-activated potassium channels in parathyroid cells by means of patch clamp techniques. We found that these channels are similar to classical calcium-activated potassium channels in the calcium requirement for activation, in the magnitude of single-channel conductance, and in selectivity between potassium and sodium. These channels are very different from the classical channels, however, in that they tend to close when the internal calcium concentration is increased above about 150 nM. This tendency leads to a region of the dose-response curve in the physiological range where increased calcium reduces the probability of channel opening, in agreement with the model as extended above. Although this result applies to the plasma membrane of parathyroid cells, it is likely to apply to the vesicle membranes as well, since the two membranes fuse during secretion.

Another approach that we are using in testing the model is to determine the effect of selectively blocking calcium-activated potassium channels. One blocking agent we tried is nonyl-triethylammonium (9-TEA), which we injected into the presynaptic nerve terminal of the squid giant synapse. Although 9-TEA

did block transmitter release, it also blocked calcium channels, thus impeding the flow of calcium into the terminal. Since the action on calcium channels would be expected to block release, these experiments did not provide a test of the model. We are still seeking a selective blocking agent for this type of test - one that blocks calcium-activated potassium channels and does not block calcium channels.

Another question regarding the role of calcium in secretion is how calcium channels in the plasma membrane of secretory cells are regulated. We have addressed this question by examining calcium currents in rat intermediate nucleus cells. Two separate calcium current components were observed. One component is transient and inactivates in less than a tenth of a second. The other component can continue for minutes, but has a component of voltage-dependent inactivation in the physiological range. This provides a mechanism for regulation of secretion, since the magnitude of the sustained calcium current depends on the detailed previous history of membrane potential as well as on the properties of the action potential.

We are also trying to determine the mechanism for the increase of free calcium in egg cells following fertilization. We have previously shown that injection into sea urchin eggs of a soluble fraction of sea urchin sperm can trigger the processes leading to the increase of calcium. We are now trying to determine the active component of this fraction. So far, we have found that the active component is a protein, that it is heat-stable, and that its molecular weight is about 20 kD.

Another question we addressed is the cause of the pathological neural responses to reperfusion and to increased oxygen tension. We have previously shown that nervous tissue does undergo oxygen-induced changes in function. We have now examined some of the properties of microglia in order to determine the role that these cells might play in causing pathological responses. We found that microglia can secrete the superoxide radical ion. This can lead to production of hydrogen peroxide and the hydroxyl free radical, agents which have been linked to cell damage and reperfusion injury. We also found that microglia can produce an amyloid-like material, and we are now trying to determine whether this material is similar to the amyloid found in the plaques of patients with Alzheimer's disease.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02087-14 LB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: W. J. Adelma, Jr., PhD. Chief LB, NINCDS

COOPERATING UNITS (if any)
University of Minnesota (J. Fohlmeister); Marine Biological Laboratory, Woods Hole, MA (C. Tyndale, R. Mueller, R. Waltz).

LAB/BRANCH
Laboratory of Biophysics, DIR

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:
2.0

PROFESSIONAL:
1.6

OTHER:
0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this study, sodium channel molecular kinetic transitions are resolved by gating current harmonics. Gating (asymmetry) currents were obtained from voltage-clamped squid giant axons in sinusoidally driven dynamic steady-states with frequency and mean membrane potential as independent variables. Harmonic content of the records as a function of the independent variables shows three kinetic sub-processes (primary and secondary activation, and inactivation), and the number of states and values of rate constants in each sub-process. Protease-treated axons have a secondary activation gating process with two states corresponding to closed and open activation gates. The strongly voltage-dependent primary process has at least five kinetic substates which determine probability for transitions in the secondary kinetics. Flickering between open and closed states is a natural kinetic consequence. The harmonic content of records from axons untreated with protease show that inactivation gating can block three of five primary activation states, thereby substantially reducing gating current. Inactivation and primary activation appear to be coupled by reciprocal steric hindrance. A slow inactivation component has been found in the gating current. This component can be removed by perfusion with protease. The temperature dependence of the gating current has been measured. A Q_{10} between 2.5 and 5 was found which is indicative of the molecular order of the gating process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02092-14 LB
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Subcellular and Extracellular Structure Associated with Nerve and Muscle.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: W. J. Adelman, Jr., PhD. Chief LB, NINCDS		
Other: R. V. Rice, PhD. IPA Fellow LB, NINCDS		
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (A. Hodge, R. Waltz, C. Tyndale, R. Mueller); Carnegie-Mellon Univ. (R. Worthington); Case-Western Reserve Univ. (R. Lasek, S. Brady, M. Fahim)		
LAB/BRANCH Laboratory of Biophysics, DIR		
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.9	PROFESSIONAL: 3.7	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this project is to examine the subcellular and extracellular structure of nerve and muscle and relate such structure to function. <u>Electron microscopy</u> in TEM, STEM and analytical electron beam probe modes, such as EELS and EDAX, determination of proteins contributing to these structures and structural modeling are methods used in this study. The following structures are probed: 1) <u>Neuroplasmic lattice</u> , 2) <u>neurofilaments</u> , 3) <u>microtubules</u> , 4) <u>axolemma</u> , 5) <u>glial cell membranes</u> , and 6) <u>myofilaments</u> . Methods developed and used in this study are: 1) <u>Stereoscopic imaging</u> , 2) <u>optical autocorrelation</u> , 3) <u>fast Fourier transformation (FFT) of STEM video images</u> , and 4) <u>STEM video image filtering and image enhancement using reverse Fourier transformation</u> . Video imaged light microscopy is used to study living neurons in <u>differential interference contrast</u> . A new method was developed for direct visualization of <u>particle velocity</u> distribution. By viewing image pairs separated by an appropriate time interval in sequential recording of the subject, the positive or negative parallax arising from particle motion results in the binocular image of a particle being perceived as raised or lowered relative to an immobile background plane depending on its direction of movement. The degree of perceived elevation is proportional to particle speed. Using this method, measured particle movement during <u>axoplasmic transport</u> in squid axons ranged from 0.05 to 0.75 $\mu\text{m/sec}$. The dynamic behavior of filopodia in a variety of cultured neuronal cell types while attaching to substrate have been analyzed. The configurational changes, particularly branching and sliding of branch points, have been and continue to be a focus of this study with particular emphasis on specific localization of fibrous proteins such as f-actin using fluorescently-labelled phalloidin. The reversible effects of hypotonicity on the structure of filopodia as well as filopodial tension have been studied and remain under investigation.		
7 - LB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02607-04 LB
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Tissue-Cultured Invertebrate Neurons.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: W. J. Adelman, Jr., PhD. Chief LB, NINCDS		
Other: R. V. Rice, PhD. IPA Fellow LB, NINCDS		
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (R. Mueller).		
LAB/BRANCH Laboratory of Biophysics, DIR		
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.7	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The aim of this project is to culture neurons in the laboratory for use in studies of axoplasmic structure and transport. These cultured neurons are also to be used in connection with voltage clamp and patch clamp experiments of ionic channels and their conductances and gating mechanisms. A number of fluorescent immuno-assays have established that neurons, glial, and muscle cells are routinely grown from squid embryos by our method. In collaboration with Alan Hodge, we have found that filopodia of cultured cells undergo a peculiar motility involving a "slip-knot" action. This motility is also present in filopodia of mammalian cell lines but is more easily studied with squid cells. Both high contrast DIC video microscopy and scanning electron microscopy are being utilized along with fluorescent probes. This project will cease with the closing of the Woods Hole Unit of LB.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02608-04 LB
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Comparative Aspects of Ionic Conductances in Nerve and Heart Cell Membranes.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. R. Clay, PhD. Physicist LB, NINCDS		
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (R. Mueller, C. Tyndale); McGill University (A. Shrier).		
LAB/BRANCH Laboratory of Biophysics, DIR		
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 1.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is concerned with a comparative analysis of <u>ionic current channels</u> in <u>nerve and heart cell membranes</u> and the relationship of these channels to electrical activity, with a particular emphasis on <u>potassium ion channels</u> in both preparations and the effects of various <u>ionic blockers</u> on these channels. During the past year the primary experimental preparations which have been used are <u>squid giant axons</u> and <u>chick embryonic heart cells</u> . This work has focused recently on the <u>Delayed Rectifier Potassium Channel, I_K</u> , in chick heart cells. A major finding has been that measurements of this component in <u>single cells</u> using the <u>suction pipette voltage clamp</u> technique are comparable in every way to our earlier measurements of I_K in aggregates of cells using the <u>two-microelectrode voltage clamp</u> technique. This result challenges a prevailing view concerning the role of <u>potassium ion accumulation</u> in the extracellular spaces of cardiac tissue. Specifically, the loading of these spaces by potassium ions is believed to play a role in generating <u>Cardiac Dysrhythmias</u> . Our results suggest that this mechanism may not be as significant as is commonly believed.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02088-14 LB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Function and Structure of Membrane Ionic Channels		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: G. Ehrenstein, Ph.D. Research Physicist LB, NINCDS Others: K. Iwasa, Ph.D. Special Expert LB, NINCDS M. Jia, M.S. Visiting Associate LB, NINCDS N. Moran, Ph.D. Visiting Associate LB, NINCDS		
COOPERATING UNITS (if any) Weed Science Laboratory - AEQI, Dept. of Agriculture, Beltsville, MD. (C. Baire and C. Mischke) University of Connecticut (R. Satter)		
LAB/BRANCH Laboratory of Biophysics, DIR		
SECTION Section on Molecular Biophysics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.3	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have previously shown that the gating of pairs of sodium channels in <u>neuroblastoma</u> cells is <u>anti-cooperative</u>; i.e., the opening of one channel reduces the probability that the other channel will open. Our analysis indicates that this type of channel anti-cooperativity does not significantly affect the values of parameters, such as channel density or rate of channel opening, that can be obtained from noise spectra. In another type of experiment, we have previously shown that cells of the plant <u>Samanea saman</u> contain <u>potassium channels</u> and that the properties of these channels are consistent with a role in opening of plant leaflets. Experiments performed with varying concentrations of external potassium demonstrated a shift in the threshold for activation. When external potassium was varied from 1 mM to 50 mM, the threshold shifted about 60 mV in the depolarizing direction. The magnitude and direction of the shift are consistent with the view that there is a <u>negative fixed charge</u> on the membrane and that potassium ions can screen this charge.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02218-12 LB												
PERIOD COVERED October 1, 1986 to September 30, 1987														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Affect of Drugs on Voltage-Dependent Ionic Conductance in Membranes														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P.I.:</td> <td style="width: 55%;">D .L. Gilbert, Ph.D. Research Physiologist</td> <td style="width: 30%;">LB, NINCDS</td> </tr> <tr> <td>Others:</td> <td>E. F. Stanley, Ph.D. Senior Staff Fellow</td> <td>LB, NINCDS</td> </tr> <tr> <td></td> <td>M. Jia, M.S. Visiting Associate</td> <td>LB, NINCDS</td> </tr> <tr> <td></td> <td>T. Behar, B.S. Microbiologist</td> <td>LMG, NINCDS</td> </tr> </table>			P.I.:	D .L. Gilbert, Ph.D. Research Physiologist	LB, NINCDS	Others:	E. F. Stanley, Ph.D. Senior Staff Fellow	LB, NINCDS		M. Jia, M.S. Visiting Associate	LB, NINCDS		T. Behar, B.S. Microbiologist	LMG, NINCDS
P.I.:	D .L. Gilbert, Ph.D. Research Physiologist	LB, NINCDS												
Others:	E. F. Stanley, Ph.D. Senior Staff Fellow	LB, NINCDS												
	M. Jia, M.S. Visiting Associate	LB, NINCDS												
	T. Behar, B.S. Microbiologist	LMG, NINCDS												
COOPERATING UNITS (if any) Georgetown University, Washington, D.C. (C. Colton); NMRI, Bethesda, MD (J. Colton); CNRS, Marseille, France (L. Fagni).														
LAB/BRANCH Laboratory of Biophysics, DIR														
SECTION Section on Molecular Biophysics														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 2.2	PROFESSIONAL: 2.0	OTHER: 0.2												
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Experiments have been performed on <u>microglia</u> , the resident macrophage in <u>central nervous system</u> . We found that these cells have the ability to secrete the <u>superoxide radical ion</u> . The amount of secretion increased with an increase in the calcium concentration. Using the patch clamp technique, a calcium channel was found. The microglia can also produce an <u>amyloid-like material</u> .														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02526-06 LB									
PERIOD COVERED October 1, 1986 through September 30, 1987											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Gated Ionic Channels in Membranes</u>											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">E. F. Stanley, Ph.D.</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 15%;">LB, NINCDS</td> </tr> <tr> <td>Other:</td> <td>J. T. Russell, Ph.D.</td> <td>Research Chemist</td> <td>LNN, NICHD</td> </tr> </table>			PI:	E. F. Stanley, Ph.D.	Senior Staff Fellow	LB, NINCDS	Other:	J. T. Russell, Ph.D.	Research Chemist	LNN, NICHD	
PI:	E. F. Stanley, Ph.D.	Senior Staff Fellow	LB, NINCDS								
Other:	J. T. Russell, Ph.D.	Research Chemist	LNN, NICHD								
COOPERATING UNITS (if any) LNN, NICHD											
LAB/BRANCH <u>Laboratory of Biophysics, DIR</u>											
SECTION <u>Section on Molecular Biophysics</u>											
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland 20892</u>											
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.8	OTHER: 0.1									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) Human subjects</td> <td>☐ (b) Human tissues</td> <td>☒ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td colspan="2"></td> </tr> </table>			☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Calcium currents were examined in rat intermediate nucleus cells. Two main currents were identified, a transient current and a sustained current. The transient current inactivated over a few tens of milliseconds. The sustained current required seconds to minutes to inactivate, but the inactivation rate was significant even at normal resting potentials. The main conclusion of this study is that any influence on the cell that leads to a sustained change in the resting membrane potential will have a significant effect on the number of activatable calcium channels. Thus, the influx of Ca^{2+} ions depends not only on the amplitude and duration of the action potential, but also on the short term history of the cell membrane potential.											

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02606-04 LB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical Transmission at the Squid Giant Synapse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. F. Stanley, Ph.D. Senior Staff Fellow

LB, NINCDS

Other: J. T. Russell, Ph.D. Research Chemist

LNN, NICHD

COOPERATING UNITS (if any)

LNN, NICHD

LAB/BRANCH

Laboratory of Biophysics, DIR

SECTION

Section on Molecular Biophysics

(project transferred from Section on Neural Membranes)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.3

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The model of exocytosis based on the existence of ion channels in the secretory vesicle membrane, that we proposed, was tested at the giant synapse of the squid. The ion-channel-blocking agent nonyl-triethylammonium (9-TEA) was injected directly into the presynaptic nerve terminal in an attempt to inhibit exocytosis. Transmitter release was blocked by 9-TEA but this effect was attributed to a blocking action on calcium channels, invalidating this approach with this compound. Further attempts at this experimental strategy will have to await more specific blocking agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02609-04 LB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Egg Activation Following Fertilization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. Iwasa, Ph.D.	Special Expert	LB, NINCDS
Others:	G. Ehrenstein, Ph.D.	Research Physicist	LB, NINCDS
	J. Russell, Ph.D.	Research Chemist	LNN, NICHD

COOPERATING UNITS (if any)

Emory University, Atlanta, GA (L. DeFelice)
LNN, NICHD

LAB/BRANCH

Laboratory of Biophysics, DIR

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

0.9

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously demonstrated that a fertilization membrane forms around a sea urchin egg when it is injected with a soluble spermatozoa fraction isosmotic with seawater. Work is proceeding to purify the sperm extract. So far, we have identified the effective component to be a heat-stable protein with molecular weight about 20 kD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02709-02 LB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Secretion of Neurotransmitters and Hormones		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Others:	G. Ehrenstein, Ph.D. E. F. Stanley, Ph.D. M. Jia, M.S. S. Pocotte, Ph.D. J. Russell, Ph.D.	Research Physicist Senior Staff Fellow Visiting Associate Staff Fellow Research Chemist LB, NINCDS LB, NINCDS LB, NINCDS LB, NINCDS LNN, NICHD
COOPERATING UNITS (if any) LNN, NICHD		
LAB/BRANCH Laboratory of Biophysics, DIR		
SECTION Section on Molecular Biophysics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.8	OTHER: .2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We performed patch clamp experiments on bovine <u>parathyroid cells</u> in an attempt to explain the following unusual properties of these cells: (1) In contrast to other <u>secretory cells</u>, where the rate of secretion increases with increasing calcium concentration, the rate of hormonal secretion in parathyroid cells decreases when the external calcium concentration increases. (2) In contrast to other cells, parathyroid cells depolarize when the external <u>calcium</u> concentration increases. We found an unusual type of <u>calcium-activated potassium channel</u> in the plasma membrane of parathyroid cells - channels that tend to close when the internal calcium concentration is increased above about 150 nM, but which otherwise behave like the usual calcium-activated potassium channels. The unusual calcium-activated potassium channels we found can account for (2) above, since an increase in external calcium concentration causes an increase in internal calcium concentration, and this leads to closing of these unusual channels and consequent depolarization. These same channels together with the model for secretion that we presented last year might also account for (1) above, provided the channels were present in <u>vesicle membranes</u> as well as the plasma membrane of parathyroid cells. In order to test this point further, we are setting up a <u>permeabilized</u> cell system to determine the dependence of parathyroid secretion on internal calcium concentration over a wide range of concentrations.		
15 - LB/DIR		

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and
Stroke

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This has been a year of remarkable new discoveries and advances in our laboratory's investigations of chronic brain diseases. We have extended our generalization that the deposition of amyloid in the brain in the form of intraneuronal neurofibrillary tangles (NFTs) and extracellularly in the form of amyloid plaques and cerebrovascular (congoophilic) amyloid deposits, is based on the conversion of a portion of host precursor protein to the beta-pleated configuration of amyloid. It is then deposited in these different patterns as insoluble fibrils, resulting from this post translational modification of the amyloid precursor protein. We have further extended our generalization that this is the same amyloid deposition not only in Alzheimer's disease (AD), aged Down's syndrome, and the normal aging brain, but also to the amyloid of the paired helical filaments (PHF) in the NFT of Guamanian amyotrophic lateral sclerosis-parkinsonism dementia (ALS-PD).

We have isolated, cloned, characterized, and chromosomally localized on the 21X-22Y region of chromosome 21, the cDNA encoding precursor protein of brain amyloid. We have found this to be a highly conserved gene from lobster to mouse and man. We have estimated the dosage of the amyloid β protein gene in human leucocytes and found three copies of the gene in sporadic AD, as well as in trisomic and karyotypically normal DS, indicating a microduplication of the 21X-22Y region of chromosome 21 in these AD patients who also have similar triple doses of adjacent genes: superoxide dismutase, protooncogene *ets-2*, and phosphofructokinase. If this is borne out for other cases of AD, the problem of its causation will resolve into one very similar to that for DS, namely, the cause of miniduplication of these specific region of chromosome 21 during gametogenesis. It must be a prezygotic event, since the duplication is present in brain cells, leucocytes, and other tissues. In Down's syndrome in mice where the duplication is on chromosome 16, we have also localized this gene in a miniduplication associated with SCD and *ets-2*. We are attempting to localize this gene in *drosophila*, anticipating that it may be on a "hot spot" for miniduplication, such as studied by Curt Sern and his group decades ago. We are attempting to get multiple doses of the gene into transgenic mice.

Further, we have used the in situ hybridization techniques and discovered that amyloid β protein mRNA is expressed differently in subsets of neurons in the hippocampus and elsewhere, and is particularly abundant in large pyramidal cells of layers III and V of the frontal cortex, which are particularly vulnerable to loss in aging and AD and to deposition of NFT's. Specific mRNA also appears in microglia, oligodendroglia and cerebrovascular endothelial cells, indicating that extracellular amyloid plaque cores and amyloid deposition in vessel walls may have an origin in these cells and not only from neurons which are affected with PHF formation in NFTs.

It may well be our previous generalization is true that interference with axonal transport underlies all these processes. Such interference leads to pooling, stagnation, or piling up of cytoskeletal elements in neurons which may precede the formation of amyloid deposits from the amyloid precursor protein which is pooled along with previously recognized cytoskeletal proteins such as neurofilament proteins and MAP-2 and MAP-tau.

The amyloid plaques of the transmissible virus dementias (kuru, CJD and Gerstmann Sträussler Syndrome, scrapie, and chronic wasting disease of mule deer) are formed by a similar virus-induced process from a different host precursor protein coded on chromosome 20 in man. We may well be observing a process of protein secondary configurational alteration which is self-replicating with properties resembling "viruses", which are responsible for autocatalytic, patterned degradation of host precursor proteins to amyloids. At times, as in

Guamanian ALS-PD and in some cases of AD, mineral deposition (especially of Si, Al, and Ca) may be involved as nucleating agents for this patterned degradation or crystallization of insoluble amyloid.

Our laboratory has also been at the forefront of demonstrating retroviruses in chronic encephalomyelitic disease, especially in the case of the oncornavirus HTLV-I, and the lentivirus HIV of AIDS. We have demonstrated the etiological role of HTLV-I in Jamaican neuropathy and tropical spastic paraparesis in Colombia and stimulated worldwide inquiry into this newly recognized slow virus infection of the brain and spinal cord. This all follows logically from our first demonstration twenty years ago of retroviruses of the spumaviridae subfamily (foamy virus group) in all normal chimpanzee brains and of the oncornavirus group in normal gibbon brains.

Child Development Project

These studies underlie and have for over three decades provided the source, motivation, clinical and biological specimens for the discoveries of slow virus infection of man, of the unconventional viruses of kuru and CJD, and of the recognition of transmissible and nontransmissible amyloidoses of the brain as the problem underlying Alzheimer's disease, aged Down's syndrome, normal aging brain, and ALS-PD in the Western Pacific. They have led to the recognition that there are environmental causes of these sometimes genetically mediated phenomena. They have provided the major impetus for studies on Ca, Al, Si deposition in the brain. They have led to our research on retroviruses, HTLV-I, and HIV of AIDS, as the cause of chronic CNS disease. They have led to our work on cerebral cysticercosis, chronic viral infections causing epilepsy, congenital CNS defects associated with endemic goiter, and our worldwide studies on Hantaan virus (hemorrhagic fevers with renal syndrome) infections. Thus, we continue to pursue the natural experiments in isolated groups as a major source of discovery of much wider implications to basic biomedical research and neurobiology than to the limited problems they seem to be at the inception of these investigations.

Slow Unconventional Viruses Causing Transmissible Brain Amyloidoses

Our laboratory has concentrated its main effort on elucidating the relationship between the proteins specific for kuru, Creutzfeldt-Jakob disease (CJD), scrapie and their host-specified precursors. It is now evident that scrapie is a protease cleavage product of a 33-37 kDa precursor, which is present in normal and infected brain tissue, but modified in the latter into a less soluble protease-resistant form. Infectivity has not been definitively associated with the 33-37 kDa "prion protein" (PrP 27-30) assemble in vitro into congophilic, birefringent rods resembling the scrapie associated fibrils (SAF) of Merz, but in so reassembling there is no restoration of infectivity.

It remains to be proven whether this normal host protein modified by scrapie virus infection is, itself, the infectious agent (an amyloid molecule patterning autocatalytically the modification of host protein precursor into its infectious autocatalytic form), or is hidden in the infectious preparations of SAF; there still remains the possibility of a very small nucleic acid fragment contributing a more conventional virus-like factor to the replication process. No non-host nucleic acid has been demonstrated, even in highly infectious preparations, and the improbable conjecture that the entire infectious process is one of an autocatalytic modification of a protein precursor and a nucleating or seeding event leading to a patterned crystallization in the form of amyloid fibers now appears likely. The host gene specifying the 33-37 kDa precursor protein has been fully sequenced.

Polyclonal and monoclonal antibodies prepared against synthetic polypeptides of the N-terminus of the amyloid of scrapie reveal varying

distribution and patterns of the epitopes in normal and infected tissues. Such antibodies have shown reactivity to the scrapie-associated proteins and, to our surprise, to many purified proteins, including purified natural and synthetic human growth hormone. This has led to great difficulty in using the antibody technique to screen cDNA libraries. However, these antibodies to SAFs, or to the synthetic polypeptide, specifically label purified SAFs from kuru-, CJD- and scrapie-infected brains. Such SAFs are not obtainable from brains of other human neurodegenerative diseases, and thus this new immunological gold-labeling technique has been used to identify serologically the SAFs in the two patients with frozen brain available of the four patients who developed CJD from injections of contaminated human growth hormone preparations.

These immunocytochemical and molecular biological studies on the scrapie/kuru/CJD-associated proteins and their normal precursors are largely aimed at preparing them in high purity and sufficient amounts for crystallographic study, and investigation at the organic chemical level of the fine structural modification involved in the conversion of normal host-protein into amyloid fibers which appears to be the major pathogenic reaction of these diseases.

For over two decades we have carefully saved at $<-70^{\circ}\text{C}$ frozen tissue from chimpanzees and other nonhuman primates affected with the human Creutzfeldt-Jakob disease and Gerstmann-Strassler syndrome viruses, kuru virus, and scrapie, and including frozen tissues collected from cats, hamsters, guinea pigs and other animals susceptible to these viruses. These were collected and saved for eventual biochemical study when ever possible. It is now possible to process these tissues for PrP 27-30 protein, SAF's, and for the 33-37 kDa scrapie-specific protein and its precursor. As a more sophisticated study of the structure of these proteins is possible, we hope to determine from this material the contribution of the host to these subacute spongiform encephalopathy viruses or slow unconventional viruses. This we are in a unique position to do since it would take two years to over a decade for other laboratories to obtain infected brain material from a number of different species, each being inoculated with the same strain of virus.

We already have indications that there are many strains of CJD viruses. Using these tissues it is possible to answer the critical question of the relative contributions of the host and the virus strain to the pathogenesis of the disorders and the molecular structure of the virus strains. Also, specific epitopic antigenic analyses of the different strains is possible, both in comparing strains of different human origin; and comparing the same strain as it has been transmitted in different species. This sets the scene for a program involving several years of intensive molecular biology: DNA and amino acid sequencing, gene cloning, and monoclonal and polyclonal antibody epitopic analyses of the disease-specific polypeptides. The results should further open the new field which we have defined: transmissible and non-transmissible brain amyloidoses.

Non-transmissible Brain Amyloidoses of Aging, Alzheimer's Disease and Other Dementia

Amino acid sequencing of the 4 kDa polypeptide subunit of the paired helical filaments of neurofibrillary tangles (NFTs), in the amyloid plaque cores, and of amorphous amyloid in congophilic angiopathy indicates that all three pathognomonic structures of the aging brain, Alzheimer's disease, Pick's disease, progressive supranuclear palsy, late Down's syndrome, and Guamanian ALS/PD are composed of identical 4 kDa (42 amino acids) subunits. In the preparations of purified PHF from NFTs of Guamanian ALS and PD no extracellular amyloid in the form of amyloid plaques or vascular amyloid deposits were present to produce possible contamination. This 4 kDa polypeptide subunit which easily

associates into dimers, tetramers, octamers, and hexadecamers, is completely different from that found in the transmissible cerebral amyloidoses.

In our last annual report we stated that "we are on a quest for the gene that encodes for the precursor of this amyloid by using a synthetic oligonucleotide probe" and further that "we are thus in a competitive race with other laboratories in trying to find this precursor." We won the race! In a series of papers that have appeared in *Science* (Feb. 20, March 13, July 3), Proceedings of the National Academy of Science, USA (April, and in press), and in Biophysical and Biochemical Research Communications we have reported the isolation, cloning characterization, chromosomal localization, and the dose by *in situ* cDNA hybridization studies for this precursor. To our surprise we find this β -amyloid protein gene to be duplicated on the long arm of chromosome 21 along with super oxide dismutase (SOD) and protein ets-2 as in Down's Syndrome. Since antibodies to the synthetic polypeptides react with many other proteins, the antibody technique of locating the gene has proved unsuccessful. We have therefore synthesized a DNA probe of 57 nucleotides, in order to use it for selecting the gene from total cellular DNA and from brain cDNA libraries by hybridization. The sequence selected for this synthetic probe has been so chosen as to be unusual in the cDNA library. A similar approach using smaller probes and less carefully selected regions has already failed in the hands of some of our colleagues, and we are thus in a competitive race with other laboratories in trying to find this precursor.

The Carbohydrate of Glycosylated Amyloid Subunits

In addition to this major thrust of work in our laboratory, which involves molecular biology, genetics, immunology, microbiology, and biochemistry, we are also collaborating with H. Kobata's Laboratory of Carbohydrate Chemistry in Tokyo in an investigation of the carbohydrate moiety on the subunit of amyloid in the 27-30 kDa protein of kuru/scrapie/CJD which is heavily glycosylated. Many of our antibodies are directed against the sugar component rather than against the amino acid polypeptide.

In Vitro Production of Amyloid Fibrils

We have been studying the re-coiling of polypeptide chains into different fine structural configurations which permit beta-pleated sheet stacking into fibrils resembling the amyloid fibrils of human pathology. Thus, using a beta-2-microglobulin we have succeeded in producing fibers that have the congophilic and green birefringent properties of amyloid fibrils and also the electron microscopic appearance of such fibers and even paired twisted fiber structures. More specifically, we have produced amyloid-like fibers from potential precursors to brain amyloids, such as the 200 kDa protein of neurofilament, and are not trying with MAP-tau. The 200 kDa neurofilament protein assembles *in vitro* into amyloid like filaments which are both congophilic and green birefringent and in morphology resemble amyloid fibrils; the amyloid-like properties increase on partial cross-linking with paraformaldehyde fixation.

The possibility that the fine structural changes of the aging or disease brain may be reproduced *in vitro* is obviously intriguing.

Neurofilament Pathology in Human Neurodegenerative Disease

By using monoclonal antibodies directed against neurofilament polypeptides, we have demonstrated an aberrant accumulation of the phosphorylated form of the 200 kDa subunit within spinal neurons and pyramidal neurons in the hippocampus and cerebral cortex of patients with Alzheimer's disease, parkinsonism-dementia, ALS and CJD and in Guamanian ALS/PD. Neurites in the senile plaques are also stained. The immunocytochemical staining is

particularly prominent in neurons bearing neurofibrillary tangles. Small amounts of congophilic amyloid can be identified in the zones corresponding to the neurons positive from the phosphorylated 200 kDa subunit. By contrast, areas having "ghost neurons" filled with congophilic amyloid are usually negative on immunostaining.

These observations suggest that there may be a relationship between the post-translational modification of neurofilament proteins, such as phosphorylation, assembly and transport. These alterations appear to be one of the common pathogenic mechanisms in the evolution of various neurodegenerative disorders, especially those associated with neurofibrillary tangles. Just what relationship this pooling of neurofilament has to the formation of amyloid fibers from the β -amyloid polypeptide, a post-translational modification of the normal amyloid precursor protein, we do not presently know.

Creutzfeldt-Jakob Disease and Human Growth Hormone

A further area of intense involvement of our laboratory has been in the problem of Creutzfeldt-Jakob disease developed in recipients of human growth hormone prepared from pooled autopsy pituitary glands by the NIH and other programs. At least four different batches of growth hormone have been contaminated, since one patient occurred in England and one in New Zealand, where none of the American products were used, and no batch of hormone was used by all five American patients, although several shared some batches. Incubation periods have ranged from 4 to 15 years, and in two cases with frozen brain we have been able to demonstrate both the PrP 27-30 protein by using our laboratory's developed ELISA test and to demonstrate with immune electron microscopy using gold bead associated antibody that the SAF preparations were specifically antigenically related to kuru, scrapie, and CJD virus. Moreover, in an additional HGH patient with motor neuron signs who was suspected having CJD, failure to detect the protein provided strong evidence against the diagnosis of CJD, and verified the subsequent neuropathological examinations showing ALS. Primates have been inoculated with more than 50 separate batches of HGH, but incubation periods are a year or more. Intense surveillance is under way of some 40,000 other young people who received the hormone injection, and one further probable case has been identified.

Tubulovesicular Particles in Brains of Animals with Spongiform Encephalopathies and their Possible Relationship to Scrapie-Associated Fibrils

A simple method was devised for negative staining of brain infected with the agent of scrapie for transmission electron microscopy. Brains of infected hamsters contained large masses of abnormal tubulofilamentous structures with irregular fuzzy surfaces. Brains of mice infected with the Creutzfeldt-Jakob disease agent contained similar tubulofilaments in smaller numbers. The abnormal tubulofilaments resembled but were readily distinguished from normal microtubules. When grids were soaked in sodium dodecylsulfate the abnormal tubulofilaments were found in various stages of fragmentation, an outer coat appearing to strip from the surface to reveal thinner fibrillary structures resembling scrapie-associated fibrils (SAF). The unmasked fibrils were identified as SAF by immuno-gold labelling, while the larger tubulofilaments were not labelled. The findings indicate that in infected brain tissue SAF may occur as an internal part of a larger structure that is disrupted by detergent and are not likely to be an artifact formed during extraction procedures. We postulate that the tubulovesicular structures seen in negatively stained preparations may be the same as the smaller, but are morphologically similar to structures that we have consistently observed over the past 20 years in thin sections of plastic-embedded brain tissues.

Diabetes Mellitus in Hamsters with Scrapie

Hamsters with experimental scrapie had abnormally elevated plasma glucose levels and reduced insulin responses after intraperitoneal injection with a standard dose of glucose. The pancreas of the infected animals had normal histology with no alteration in distribution of cells secreting insulin, glucagon and somatostatin hormones. Plasma cortisol levels were not elevated in animals with scrapie which militates against stress being the underlying mechanism for hyperglycemia. Scrapie-induced diabetes mellitus in hamsters may be mediated through damage to the central nervous system.

Inactivation of the Agents of Scrapie and Creutzfeldt-Jakob Disease on Surfaces

The agents of spongiform encephalopathies are notoriously difficult to inactivate. Three regimens have often been recommended to disinfect contaminated materials: exposures to sodium hypochlorite, sodium hydroxide, or the steam autoclave. However the studies on which those recommendations were based were performed with aqueous suspensions of infected tissue. In disinfection of conventional viruses it is generally accepted that such "liquid dilution" methods should be supplemented by studies more closely approximating the conditions under which disinfectant or sterilization will be performed in actual practice—"use-dilution" or "simulated-use" methods. We conducted simulated-use studies of the inactivation of the agents of scrapie and Creutzfeldt-Jakob disease (CJD) on surfaces. They suggest that currently recommended disinfectant regimens may not be completely effective.

A modification of the "glass-crushing" method of Chen was employed to study inactivation by heat and a variety of disinfectants on the agents of hamster-adapted scrapie agent and mouse-adapted CJD agent.

Preparations of scrapie agent displayed progressive reduction in infectivity after storage at -70 C. Only freshly prepared surfaces were used in later experiments. As in previous studies in liquid dilution, none of 9 commonly used disinfectants consistently freed glass objects of scrapie infectivity after 10-minute exposures; potassium permanganate gave irregular results, as before. Sodium hypochlorite reduced both the number of objects on which residual infectivity was demonstrated and the number of animals infected but never removed all of the agent, even after exposure for an hour. Calcium hypochlorite had no obvious effect. Sodium hydroxide 1N, known to reduce titers of scrapie and CJD agents in liquid dilution markedly, freed only 20% to 80% of scrapie-contaminated surfaces of infectivity; 2N NaOH and KOH apparently disinfected all glass objects contaminated with scrapie agent and gave comparable results with the CJD agent. Ammonia water was ineffective.

Although heat reduced titers of infectivity of the scrapie agent markedly and promptly, a small fraction proved very resistant. Even the steam autoclave at 132°C apparently failed to remove all infectivity from glass surfaces contaminated with the scrapie agent.

In short, scrapie and CJD agents are more difficult to inactivate completely when dried onto surfaces than in liquid dilution. Previously recommended regimens of heat sterilization left residual infectivity on the surfaces. 2 N NaOH and KOH appear to be more effective disinfectants than the other methods tested.

Toward a Biochemistry of Silicon and Aluminum

The metabolic adjustment to severe environmental deficiency of calcium and magnesium which is responsible for the deposition of calcium, aluminum, silicon, phosphorous and other minerals in brain cells in early life in the high incidence foci of amyotrophic lateral sclerosis (ALS) and parkinsonism dementia (PD), and the early appearance of Alzheimer's neurofibrillary tangles (NFTs) in isolated populations in the Western Pacific (Guam, Japan, West New Guinea), was first suggested by epidemiological and ecological studies. Minerals analyses of environmental specimens of soil and water confirmed this hypothesis. Finally, electron probe X-ray analyses using both energy dispersive and wave length dispersive spectroscopy has demonstrated these long term deposits in the brains of Guamanian ALS and PD patients, and in normal individual exposed to the same environmental deficiencies, to show the early appearance of NFTs. When these Ca^{++} and Mg^{++} deficiencies are removed by increased access to outside foodstuffs, changed water supply, improved transportation and economy, all three diseases (Alzheimer's NFTs, ALS and PD) have declined markedly in incidence or disappeared within a period of two or three decades.

This discovery of the primary cause of all three pathological processes in the Western Pacific isolates has led to animal experiments which further substantiate the hypotheses (see below) and stimulated a renewed interest in the role of mineral deposition in interfering with axonal transport. Even therapeutic and prophylactic clinical regimens are now suggested and some are under study.

Furthermore, the role of silicon and its polymers in altering the secondary structure of proteins through long series of hydrogen bonds is now under investigation. Silicon and aluminum compounds can interact strongly with phospholipids, lipids, carbohydrates and oligonucleotides as well as with polypeptides. Thus, mineral deposits of montmorillonite clays--calcium-aluminum-silicates--and hydroxyapatites can denature and alter protein fine structure and conceivably play an active role in degradation of host precursor proteins to amyloids.

The recent confirmation of older observations of silicon containing deposits in the center of purified insoluble amyloid plaque cores from Alzheimer's disease patients and in Alzheimer's NFTs has greatly stimulated interest in the possible role of these silicon and aluminum containing mineral deposits as nucleating agents, or even as autocatalytic agents, in the deposition or crystallization of such amyloid deposits. The work and thinking of this laboratory in these directions has had a major impact on determining the course of modern inquiry into aging and the degenerative amyloidoses of brain, including Alzheimer's disease.

Possible Role of Low Dietary Ca and Mg And a Neurotoxin in the Evolution of Motor Neuron Disease

Oral administration of low calcium and magnesium diet to young cynomolgus monkeys (*Macaca fascicularis*) for a period of 4 years has induced degenerative changes and variable degrees of intracellular calcium accumulation in the motor neurons of the spinal cord and brainstem, and in the giant Betz cells of the cerebral cortex. Supplementation with low dose aluminum and manganese chloride has resulted in a cellular accumulation of argentophilic material of neurofilament origin in different areas of the central nervous system. None of the animals, however, showed overt clinical symptoms despite these neuropathological changes.

Immunohistochemical staining, using monoclonal antibodies against neurofilaments, has revealed an aberrant accumulation of the phosphorylated

form of the 200 kDa subunit protein within the perikarya of motor neurons in the spinal cord, mesencephalic component of trigeminal nucleus, zona compacta of substantia nigra, and of large pyramidal neurons in the cerebral cortex. This abnormal accumulation was noted maximally in animals fed aluminum and only minimally in those fed a low Ca^{++} and Mg^{++} diet alone.

In addition to the neuronal pathology, axonal spheroids were seen both in the neuropil of the nuclear area and white matter. As in ALS, the lateral and anterior columns of the spinal cord, the spinocerebellar tracts and cortico-spinal tracts in the brain stem revealed axonal swellings, spheroid formation and focal axonal loss. Gliosis was conspicuously absent.

These observations support the hypothesis that low Ca^{++} and Mg^{++} levels interfere with axonal transport of the neurofilament subunits. This is further accentuated by the addition of aluminum. It is believed that the compact, relatively rigid molecules of phosphorylated 200 kDa neurofilament proteins accumulate in the neuronal soma leading to functional derangement and eventually to cytolysis.

This nonhuman primate model provides a means to understanding the pathogenic mechanism involved in the evolution of lesions in motor neuron disease.

Retrovirus Encephalomyelopathic Human Lentivirus (AIDS) in Children and Adults

Our laboratory is also working on the problem of the primary encephalitis which characterizes almost all cases of childhood AIDS acquired congenitally from a human immunodeficiency virus (HIV) infected mother. The HIV mothers are giving rise to infected babies in 80% of their offspring, and some 80% of these infected offspring develop clinical AIDS. Most develop disease within one to two years after birth; a few are delayed as four to five years of age. All, however, develop a primary encephalitis characterized by dysarthria, speech impairment, with eventual aphasia, and severe midline truncal ataxias and loss of developmental milestones.

We have demonstrated the virus in the brains of these infants by *in situ* DNA hybridization, by fluorescent antibody localization, and by electron microscopy. In histological studies we have demonstrated that there is a specific neuropathology with large, multinucleated macrophages in the brain, loaded with virus particles that are visible by electron microscopy. Similarly, there are brain cells which appear to be astrocytes, bulging with the human lentivirus particles. In neurons, these virus particles are rarely seen, and when seen are few in number. It was from the awareness of the primary human lentivirus encephalopathy of infants and children that a search was made in the brains of adults, and similar pathology was found in more than half of the fatal cases of AIDS.

It is only in the last two years, therefore, that clinicians have become increasingly aware that many adult AIDS patients show varying degrees of dementia which is not due to opportunistic infection with mycoplasma, mycobacterium, yeast, toxoplasma, cytomegalovirus, or herpes simplex virus.

The human lentivirus has thus become the major cause of encephalitic death among children and adults in the United States. In adults and more frequently in children, primary encephalitis from human lentivirus infection may occur without an immune deficiency syndrome. Thus, we are dealing with another example of transmissible virus infection resulting in a chronic dementia.

Search for an Animal Model of AIDS

Our laboratory first demonstrated active infection of chimpanzees with LAV and HTLV-III and with primary human tissues obtained from AIDS patients. The animals become seropositive but do not develop clinical disease, and if there is

any alteration in immune function, it is a transient lymphocytosis with moderate impairment of lymphocyte function, but not a helper-suppressor ratio change equivalent to that in human AIDS. The animals show no clinical disease three years after inoculation but they remain seropositive and viremic.

Such chimpanzees developing primary infection on inoculation with human brain tissue from AIDS patients provided the first demonstration of the live virus in the brain of AIDS patients. Since such infection has occurred even at high dilutions of suspensions of brain tissue from AIDS, the presumption is that the virus is in brain and in considerable quantity.

Many other species of nonhuman primates have been inoculated without obtaining disease, primary infection, or antibody conversion; however, an occasional rhesus monkey inoculated with these human viruses do not develop a fatal encephalopathy equivalent to that caused by HTLV-III. The human lentiviruses do not produce disease, even though they are very closely related to HTLV-III. Thus, we are without a good experimental model for vaccine evaluation in small animals or in nonhuman primates, and all that can be done at present is to test for the ability of vaccines to protect against primary infection.

Our laboratory first introduced the studies of the Icelandic visna and maedi sheep diseases in the United States at the NINDB Symposium on Slow Viruses in 1962, to which the Icelandic workers were invited as participants. We made the first isolations in the United States of the visna virus, later defined as the prototype lentivirus, from the brain of a sheep with Montana sheep disease. The maedi virus previously was thought to be causing only pulmonary involvement in Montana sheep disease. It is now known to be the same virus as visna, which may cause maedi, or zoegersiekte, in Iceland and the Netherlands, respectively, the pulmonary forms of visna virus infection.

AIDS virus (or HIV) belongs to this group, now called the lentiviruses. The entire group has been named a lentivirus sub-group of the Retroviridae, and the AIDS viruses are the first human representatives of the group, which contains visna virus, equine infectious anemia virus, and caprine arthritis encephalitis virus. We have found that horses inoculated with the human AIDS lentivirus develop a transient antibody response, but no disease develops.

Further Studies of Chimpanzees with Asymptomatic Persistent Infections with Human Immunodeficiency Virus

Two of 11 chimpanzees inoculated intracerebrally and intravenously with suspensions of brain tissue from patients with AIDS encephalopathy acquired persistent infection as did 20 of 23 animals inoculated with cell-culture isolates of HIV, including one of 2 inoculated with strains of HIV from human brain. No HIV-infected chimpanzee has developed a neoplasm, unequivocal opportunistic infection or other overt evidence of immunodeficiency, nor has any showed unequivocal clinical signs of encephalopathy during observation periods of 15 to 42 months.

One other chimpanzee that had received HIV-infected cell-culture fluid 3 months previously, developed a transient illness. A routine intradermal tuberculin test, performed 23 months postinoculation, resulted in a severe positive reaction. Physical examination was unremarkable except for olive-sized inguinal lymphadenopathy. T-cell helper/suppressor ratios and lymphoproliferative responses to mitogens were normal. Diagnostic procedures, including abdominal and thoracic radiographs, performed to determine whether the animal had tuberculosis were not remarkable. A sample of gastric lavage, however, revealed *Mycobacterium avium* intracellular (MAI). Other cultures for acid-fast bacilli of urine, blood, bone marrow, and a lymph node biopsy were negative.

While in quarantine the animal exhibited episodes of unusual behavior characterized by self-mutilation, screaming, and turning rapidly about in its

cage. This behavior subsided temporarily when a second chimpanzee was placed in an adjoining cage, but three months later bouts of violent behavior, some requiring treatment with tranquilizers, recurred. Tremors of the trunk resembling shivering were also noted. The animal lost 5 kg of weight. After the second two-week episode of abnormal behavior, the chimpanzee improved again, gained weight and is now normal. Repeated lumbar punctures during and after the episodes of abnormal behavior revealed only variable numbers of cells, usually fresh erythrocytes, normal total protein content, low levels of IgG antibodies to HIV, and no virus or p25 viral core antigens.

Another healthy HIV-infected animal was repeatedly exposed to amyl nitrite by inhalation and was inoculated intravenously with a large dose of human cytomegalovirus (CMV), a virus to which it had no antibodies. (Both nitrite abuse and infections with various viruses, including CMV, have been proposed as cofactors that might provoke the transition to AIDS in asymptomatic humans infected with HIV.) The animal tolerated both the nitrite and exposures without ill effects and has no sign of AIDS. It acquired high levels of serum neutralizing antibodies to CMV.

CSF of 4 of 8 other animals tested contained ELISA antibodies in low titers (highest 1:26). Antibody indices were calculated by the method of Link to correct for serum to CSF leak. Fluids of two animals had antibody indices higher than 1. In humans antibody indices greater than 1 are suggestive, and those greater than 2 are highly suggestive of intrathecal antibody synthesis. However, normal chimpanzees in our colony have higher CSF albumin and lower serum albumin levels than do normal humans, resulting in CSF/serum albumin ratios in chimpanzees that are very high by human standards. Even in humans the normal range for antibody indices has not been determined for CSFs with high albumin ratios. No oligoclonal bands of globulin were found in any CSF. No virus was isolated from CSF. No viral antigen was detected in CSF by ELISA.

Because the changes in CSF were equivocal we decided that it was justified to examine brain tissue from one of the chimpanzees. The chimpanzee with the second highest level of CSF antibody was selected for brain biopsy. (The animal with the highest level was not suitable because it had very thick brows.) An open biopsy of the right frontal lobe was performed under general anesthesia by an experienced neurosurgeon.) No histopathological changes were found; there were normal meninges and cortical parenchyma with no inflammation and no giant cells. Neuronal and glial nuclei were normal in number and morphology. Cerebral white matter was also completely normal with no evidence of the pallor often seen in brains of humans with AIDS encephalopathy. No viral core antigens were detected in cortex or underlying white matter by alkaline-phosphatase-anti-alkaline-phosphatase immunostaining, and no viral RNA was detected by *in situ* hybridization. No virus was isolated from the brain cultivated in H9 cells for 90 days, although HIV was isolated from blood obtained from the animal at the time of biopsy. The animal is neurologically normal 40 months after infection with HIV and 15 months after biopsy.

In conclusion, there is still no clear evidence that the brain is involved in persistent HIV infections of chimpanzees, and no animal has developed AIDS, including one treated with nitrite and infected with CMV. At least one animal has an infection with MAI, but it is not known whether that represents an opportunistic infection in an otherwise healthy chimpanzee. Low levels of antibodies to HIV were detected in CSF of some animals. We speculate that those antibodies may reflect the early transient invasion of the CNS in chimpanzees that occurs in primary HIV infections of humans. Continued observation of persistently infected chimpanzees is required to learn whether some of them ultimately develop AIDS or AIDS encephalopathy.

Human T-cell Lymphotropic Viruses (HTLV-I) in Transverse Myelitis and Multiple Sclerosis

We have continued our studies of Jamaican neuropathy in Jamaica, and of Pacific (tropical) spastic paraparesis in the Tumaco area of southwestern Colombia on the Pacific coast. The Tumaco focus has a very uniform disease, which Dr. Gajdusek has studied on three occasions in the field in Colombia. In the earlier studies we found that patients had a much higher percentage of treponema positive spinal fluid than control patients, but in our most recent study there has been a striking decline in the rate of positivity, probably indicating that the earlier, not the newer, patient had yaws. On the other hand, we have now demonstrated an IgG antibody response in spinal fluid and serum to the human T-cell lymphotropic virus type I. This HTLV-I antibody positive spinal fluid in the spastic paraparesis patients occur in most of them, and in none of the controls with other neurological diseases. In the coastal area the seropositive titer in normal adults is very low, less than one-tenth found in the patients. In Jamaica, where the disease occurs island-wide, the same finding has been reported by our group and we have confirmed the ELISA studies by Western blot and radioimmunoassay (RIA). We are also doing viral isolation studies on CSF from several Jamaican patients at this time; having already had an HTLV-I isolation from blood. In the study of spastic paraparesis in the Seychelles we have found similar seropositive rates for IgG antibodies to HTLV-I and the positivity rate for treponemal infection is no higher than in the controls.

New Japanese data indicates that a progressive chronic myelopathy is associated there with HTLV-I infection; thus, the findings in Martinique and our discoveries in Jamaica and the Colombian focus have been supported by those from Japan, the Seychelles and Trinidad, and serve to emphasize the need for intensive study of neurological involvement with HTLV-I infections, particularly in view of the fact that antibodies to a virus of this group have been found in cases of multiple sclerosis (MS). The similarity to MS of some of the histopathological changes in Jamaican patients has also provoked the thought that this group of viruses could play a role in producing varied manifestations of one disease process in different ethnic groups and regions--MS in temperate climates and tropical paraparesis in the tropical and subtropical areas.

It must be emphasized that the human T-cell lymphotropic viruses types I and II are not related virologically to the LAV-HTLV-III virus which is a lentivirus. They are not lentiviruses; they belong to a totally different subgroup of the retroviruses, and it is these viruses that may be producing chronic myelopathies; therefore, the problem needs much further clarification and investigation.

We are now studying intensively MS tissues including CSF and sera for HTLV-I antibody and frozen MS brain tissues for the presence of virus subunits, using *in situ* DNA hybridization to search for copies of the virus genome and fluorescent antibody techniques to search for the presence of subunit proteins of the virus.

Antibodies to HTLV-I in Populations of the Western Pacific

Two populations from coastal provinces of Papua New Guinea (PNG) were recently reported to have high prevalence of antibodies to HTLV-I (Kazura et al., *J Infect Dis* 155:1100-1107, 1987). We studied sera from 13 other indigenous populations of the southwestern Pacific, including three from the highlands of PNG, two from islands off the northern coast of PNG, one coastal and one highland population from West New Guinea (Irian Jaya), one indigenous population from Australia's Cape York

peninsula, three from the Solomon Islands, and two from the Banks and Torres Islands. We found great variation in prevalence of antibodies to HTLV-I among the population groups.

Prevalence of IgG antibodies to HTLV-I varied from zero to 25%. In most high-prevalence populations children and adolescents as well as adults had antibodies, though percentages tended to increase with age. There were no clear cut differences among females and males in most populations. Three populations from New Guinea living in areas near the coast (Asmat, New Britain, New Hanover) had high prevalence, while two highland populations from PNG (Aseki Anga and Baruya Anga) and one from West New Guinea (Bilogai) had no antibodies. [We recently tested sera collected last year from the Hagehai people living in a mountainous area of Medang Province, a coastal province of PNG; they too had high prevalence of antibodies to HTLV-I.] A few Anga highlanders working on the island of New Britain had antibodies but with a prevalence far lower than among indigenous people. A population of native Australians from the Cape York peninsula, located near New Guinea collected 30 years ago had a low percentage of antibodies.

Residents of 2 small Solomon Islands (Anuta and Tikopia, 2 Polynesian outliers) had low prevalence of antibodies, while those of larger Rennell Island had high prevalence. Residents of Toga in the Torres and Banks Islands had a moderate prevalence of antibodies and those on Ureparapara had high prevalence.

Although the sera were obtained from subjects selected nonrandomly and at different times, the differences suggest a roughly consistent pattern. Populations with greater opportunities for outside contact generally had higher prevalence of antibodies to HTLV-I.

Viliusk Encephalomyelitis in Yakut People in Siberia, USSR

We have just published a definitive bibliography of references on this disease since most reprints are unfamiliar to English-speaking neurologists. A complete review of this disease in English has been submitted to Brain. A detailed and comprehensive report of Viliusk encephalomyelitis (VE) is being prepared reporting the unique CNS pathology.

Analysis of clinical descriptions of 248 VE cases and a comprehensive clinical characterization of the disease has been made in comparison with the results of neuropathological study of 64 cases. VE geographical distribution and epidemiological features of the disease based on high verified clinical material were also studied. A hypothesis of an infectious disease with a strong inflammatory component, probably slow virus infection, is hypothesized. Further studies on neuropathological and etiological aspects of VE have been initiated.

Hantaviruses and Hemorrhagic Fever with Renal Syndrome

Our work on the viruses of hemorrhagic fever with renal syndrome (HFRS) continues on an international level. We were the first laboratory to demonstrate the presence of a hantavirus (Prospect Hill virus) in a native American rodent and to demonstrate that the massive Chinese epidemics of the past two decades have been caused by viruses closely related to those isolated from HFRS patients in Korea and Siberia. Our involvement has also been intensive with this study of HFRS in the Balkans, particularly Yugoslavia and Hungary, and in Scandinavia, where in a milder form, called nephropathica epidemica, hundreds of cases may occur in a given year. Last year China alone had more than 100,000 cases of HFRS, with approximately 5% mortality in most centers. Over 10% of the patients develop an encephalopathy during the course of the disease, but more interestingly, the disease affects particularly the

pituitary gland and the pituitary stalk where hemorrhages are usually found in autopsied material.

During the past year we have been investigating severe fatal hemorrhagic illnesses in renal insufficiency of suspected hantavirus in the United States. A fatal case in an illegal immigrant in Texas prompted us to investigate the prevalence of Hantavirus infection in wild rodents in designated regions in Texas. A virus antigenetically distinct from other known hantaviruses has been isolated from Mus muscalus captured in Leahey, Texas. Studies are now underway to further characterize this isolate DNA and to determine its role in human infection and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER CNSS Z01 NS 01282-23
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D.C. Gajdusek, M.D.	Chief LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief LCNSS
	David M. Asher, M.D.	Research Medical Officer LCNSS
	Paul W. Brown, M.D.	Medical Director LCNSS
	Ralph M. Garruto, Ph.D.	Senior Research Biologist LCNSS
	Richard Yanagihara, M.D.	Medical Officer LCNSS
COOPERATING UNITS (if any) AUSTRALIA: Dr. Timothy Asch, Australian National University, Canberra; Dr. Cyril Curtain, CSIRO, South Melbourne; Dr. Eric French, Mt. Eliza; Dr. Chev Kidson, Queensland Institute of Medical Research, Brisbane; (continued)		
LAB/BRANCH Laboratory of Central Nervous System Studies, DIR, NINCDS		
SECTION		
INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
12	8	4
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies of human biology of vanishing primitive societies focus on neurological development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which all our studies have evolved. Techniques of molecular biology, immunology, virology, endocrinology and biochemistry and field epidemiological, clinical, linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens collected on expeditions to Micronesia, Polynesia, Solomon Islands, New Hebrides, New Guinea, Indonesia, South America, Asia and Africa are used. Studies on nutrition, reproduction, fertility, neuroendocrine influences on age of sexual maturation and aging, genetic polymorphisms, genetic distance, unusual and odd employment of the higher cerebral functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, epilepsy, spastic paraparesis, familial parkinsonism, other CNS degenerations, hysterical disorders, schizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections are investigated. Zoonoses such as hemorrhagic fever with renal syndrome in China, Japan, Korea, USSR, Scandinavia, and the Balkans are studied including these newly recognized Bunyawera viruses in the U.S. Acquired immune deficiency syndrome studied by our group in 1950-1960 have been reinitiated. Human evolution and adaptability to high altitude, excessively wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychological stress are under investigation in appropriate population isolates.		
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YUGOSLAVIA: Dr. A. Gligic, Univ. of Belgrade, Belgrade; Dr. A. Terzin, Department of Microbiology, Faculty of Medicine, Novisad; Prof. J. Vesenjak-Hirjan, Sveucilistau Zagrebu, Zagreb.

- Sub-Project I: Study of the development patterning of the human nervous system (cybernetics of human development).
- Sub-Project II: Human evolutionary studies in isolated primitive groups.
- Sub-Project III: Studies of isolated Micronesian populations.
- Sub-Project IV: Studies of isolated New Guinea populations.
- Sub-Project V: Studies of Australian Aborigines.
- Sub-Project VI: Studies of isolated New Hebrides and Solomon Islands populations.
- Sub-Project VII: Studies of Central and South American Indians.
- Sub-Project VIII: Developmental, genetic and disease patterns in primitive and isolated populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia, South and Central America, and the Arctic.
- Sub-Project IX: Experimental developmental neuropsychiatrics in infantile programming: a empirical approach to the language of information input into the nervous system.
- Sub-Project X: Ciphers and notation for the coding of sensory and motor data for neurological information processing.

- Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.
- Sub-Project XII: Studies of high incidence of neurological disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated groups (HTLV-I; AIDS; Kuru; Creutzfeldt-Jakob disease, Gerstmann-Strasser Syndrome; familial Parkinsonism; Huntington's Disease; Marie's Cerebellar Ataxia; Neurofibromatosis; cysticercosis).
- Sub-Project XIII: Studies of high incidence of non-neurological disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated groups (male pseudohermaphroditism, diabetes, progressive lung disease and asthma).
- Project Description: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (are attached)
- Publications: Listed on pages 29 - LCNSS/DIR through 32 - LCNSS/DIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00969-23 CNSS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others: Clarence J. Gibbs, Jr., Ph.D. Deputy Chief LCNSS

David M. Asher, M.D. Research Medical Officer LCNSS

Paul W. Brown, M.D. Medical Director LCNSS

Ralph M. Garruto, Ph.D. Senior Research Biologist LCNSS

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Central Nervous System Studies, DIR, NINCDS

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

24

PROFESSIONAL:

14

OTHER:

10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, Parkinsonism-dementia, Parkinson's, Pick's, and Alzheimer's disease, Huntington's chorea, supranuclear palsy, other presenile dementias, spinocerebellar ataxias, epilepsy, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, autism, SSPE, PML, dialysis encephalopathy, and intracranial neoplasm. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies: kuru and Creutzfeldt-Jakob disease (CJD) of man; scrapie and mink encephalopathy are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasingly recognized worldwide causes of death: high incidence foci, transmission by corneal transplant or brain surgery, and occupational hazards from exposure to diseased or infectious brain. In order to determine the usual mode of infection with the virus, a worldwide epidemiological study of transmissible virus dementia (CJD) cases is underway with special attention to familial clusters of cases and with a quest for possible relationship of scrapie of sheep to the human disease.

Familial and nonfamilial dementia and the dementias of senility are studied. The autoimmune responses to specific brain antigens in CNS diseases are under intensive investigation. DNA *in situ* hybridization and electrophoretic focusing partition of proteins along with enzymatic and hybridoma immunofluorescence and many other techniques are used to try to identify viral subunits and partial genomes in tissues in chronic diseases.

PRINCIPAL INVESTIGATORS: (continued)

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Minnesota--Dr. Ashley T. Haase, University of Minnesota, Minneapolis; Nevada--Dr. Warren V. Huber, V.A. Medical Center, Reno.

New Jersey--Dr. L. Epstein, New Jersey College of Medicine and Dentistry, Newark. New York--Dr. Samuel J. Ayl, The National Foundation March of Dimes, White Plains; Dr. Jordi Casals, Mt. Sinai School of Medicine, New York; Dr. Teresita S. Elizan, Mt. Sinai School of Medicine, New York; Dr. Scott Halstead, Rockefeller Foundation, New York; Dr. Asao Hirano, Montefiore Hospital, Bronx; Dr. John Hotchin, Department of Health, Albany; Dr. Imaharu Nakano, Montefiore Hospital and Medical Center, New York; Dr. Michael L. Shelanski, New York University Medical Center, New York; Dr. Roger D. Traub, IBM Thomas B. Watson Research Center, Yorktown Heights; Dr. James D. Watson, Cold Spring Harbor Laboratory, Cold Spring. Ohio--Dr. S.M. Chou, Cleveland Foundation, Cleveland; Dr. Maurice Victor, Metropolitan General Hospital, Cleveland. Texas--Dr. Samuel Baron, University of Texas, Galveston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Midland. Virginia--Dr. J. L. Hourigan, Arlington. Washington--Dr. Ellsworth C. Alvord, Jr., University of Washington, Seattle. Washington, D.C.--Dr. Harold Booker, Veterans Administration Central Office, Washington; Dr. John Kurtzke, V.A. Hospital, Washington; Dr. Frederick C. Robbins, National Academy of Science, Washington; Dr. Fuller Torrey, St. Elizabeth's Hospital, Washington. Wisconsin--Dr. Richard F. Marsh, University of Wisconsin, Madison; Dr. Gabriel Zu Rhein, University of Wisconsin, Madison.

YUGOSLAVIA: Dr. A. Gligic, Institute of Immunology and Virology, Beograd; Dr. Miha Likar, Mikrobioloski Institut, Ljubljana; Dr. D. Terzin, Institute of Virology, Serajevo; Prof. J. Vesenjaj-Hirjan, University of Zagreb, Zagreb.

- Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, presenile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.
- Sub-Project II: Characterization and pathogenesis of kuru virus.
- Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia virus).
- Sub-Project IV: Scrapie: studies on the purification, physical and biological characterization and nature of the virus.
- Sub-Project V: In vitro cultivation of the viruses of the subacute spongiform virus encephalopathies in cell cultures.
- Sub-Project VI: Host range of susceptible laboratory animals to the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project VII: Strain variations among the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project VIII: Cell-fusing properties of the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project IX: Resistance to radiation of the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project X: Resistance to disinfectants of the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project XI: Tissue and cell culture techniques used to unmask slow infection of man and animals using brain and viscera biopsy and early autopsy, bone marrow and peripheral leucocyte specimens.
- Sub-Project XII: The syncytium-forming viruses (simian and human foamy viruses).
- Sub-Project XIII: Studies on transformed human brain tissue in vitro and characterization of associated virus.
- Sub-Project XIV: Electron microscopic membrane studies of subacute spongiform virus encephalopathies.

- Sub-Project XV: Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates.
- Sub-Project XVI: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.
- Sub-Project XVII: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.
- Sub-Project XVIII: Fluorescent antibody studies on the intracellular localization and identification of virus antigens in vivo and in vitro in tissues from patients with subacute diseases of the central nervous system.
- Sub-Project XIX: Isolation and characterization of adenovirus from the urine of chimpanzees.
- Sub-Project XX: Development of serological and immunological test system for use in the study of slow infections of the central nervous system.
- Sub-Project XXI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.
- Sub-Project XXII: Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections.
- Sub-Project XXIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.
- Sub-Project XXIV: Sequential development of kuru-induced neuropathological lesions in spider monkeys.
- Sub-Project XXV: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.
- Sub-Project XXVI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia.
- Sub-Project XXVII: Study of mitochondrial mutants from scrapie-infected mouse brain cells.
- Sub-Project XXVII: Study of mitochondrial mutants from scrapie-infected mouse brain cells.
- Sub-Project XXVIII: Isolation and characterization of the etiological agent of Scandinavian nephro-nephritis epidemica.

- Sub-Project XXIX: The pathogenesis of Korean hemorrhagic fever virus and the elucidation of its biological and physical properties.
- Sub-Project XXX: Worldwide seroepidemiological evidence of antibodies in human populations to the virus of Korean hemorrhagic fever.
- Sub-Project XXXI: Development of an enzyme-linked immunoadsorbent (ELISA) test for the diagnosis and epidemiology of cystercercosis-induced epilepsy.
- Sub-Project XXXII: Studies on the cytochemical and morphological properties of neurons cultured in vitro.
- Sub-Project XXXIII: Development of immunological markers for the detection of autoantibodies to neurofilaments in the sera of patients with subacute spongiform encephalopathies.
- Sub-Project XXXIV: Studies to determine the neurophysiological changes of neurons in vitro infected with CJD.
- Sub-Project XXXV: Effects of the subacute spongiform viruses on nerve cells grown in vitro.
- Sub-Project XXXVI: In vivo and in vitro studies to determine the etiology of myasthenia gravis, Villiuis encephalomyelitis and ALS-PD in high incidence foci of the Western Pacific.
- Sub-Project XXXVII: Neurophysiological study of animals experimentally infected with subacute spongiform virus encephalopathies.
- Sub-Project XXXVIII: Studies on in vivo pathogenecity of the retroviruses related to AIDS: HTLV (Gallo); French LAV-LOISEAU virus (Montagnier)
- Sub-Project XXXIX: Attempts to transmit or isolate in vitro an etiological agent from AIDS, from pre-AIDS patients with lymphadenopathy syndrome, and from encephalitis associated with AIDS.
- Sub-Project XXXX: Isolation and characterization of "unconventional viruses" (CJD) from multiple lots of human pituitary growth hormone.
- Sub-Project XXXXI: Epidemiology of progressive degenerative disease of the CNS in recipients of human pituitary growth hormone.
- Sub-Project XXXXII: Development of procedures to exclude "unconventional viruses" from preparations of human pituitary growth hormone.
- Sub-Project XXXXIII: Preparation and characterization of synthetic polypeptides for scrapie, kuru, CJD and core protein of amyloid plaques in Alzheimer's disease.

- Sub-Project XXXXIV: Studies on the effects of altered slow axonal flow in the pathogenesis of subacute progressive degenerative disease of the nervous system.
- Sub-Project XXXXV: Studies on the deposition and distribution of heavy metals and essential minerals in central nervous system tissue from patients with progressive neurodegenerative disorders.

Project Description: Chronic Central Nervous System Disease Studies (described fully on pages 1-LCNSS/DIR through 13-LCNSS/DIR).

The projects (I through XXXXV) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications. Contractural phases of this work are being conducted at University of Southwestern Louisiana, New Iberia Research Center, New Iberia, Louisiana.

Publications: Pages 29-LCNSS/DIR through 32-LCNSS/DIR

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5. Brown, P.W., Gibbs, C.J., Jr., Gajdusek, D.C., Cathala, F., and LaBauge, R.: Transmission of Creutzfeldt-Jakob disease from formalin-fixed, paraffin-embedded human brain tissue. New England Journal of Medicine, 315:25 (December 18), 1614-1615, 1986.
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ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Experimental Neuropathology

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ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Experimental Neuropathology, DIR
National Institute of Neurological and Communicative
Disorders and Stroke

Henry deF. Webster, M.D., Chief

The Laboratory of Experimental Neuropathology (LENP) includes the Cellular Neuropathology Section (CN) and the Neurotoxicology Section (NT). The main goal of the Laboratory's research program is to investigate cellular mechanisms of myelin breakdown, especially those that are directly related to multiple sclerosis, AIDS, progressive multifocal leukoencephalopathy (PML) and other human demyelinating diseases. Other closely coordinated projects use biochemical methods to explore gangliosides and protein phosphorylation in the brain. Neurotoxic actions also are investigated. Important discoveries during the past year include: (1) Using immunohistochemical methods, HSV-2-related antigen was demonstrated in a few demyelinating plaques in a small proportion of multiple sclerosis cases that were examined. Other studies of experimental HSV-2 infection have explored early nervous system lesions after primary genital infection as well as patterns of recurrent disease after reactivation of a latent infection. (2) The development of a double-label immunocytochemical method that uses a monoclonal antibody to JC virus T-antigen and a polyclonal rabbit antibody to JC virus capsid antigens. Use of this method on sections of frozen human CNS PML cases with and without AIDS has shown that JC virus T-antigen is more widely distributed than previously thought. It is found frequently in glial cells that do not yet express capsid antigen and are located in more normal appearing white matter around lesions. (3) Two novel protein kinases have been discovered that can be regulated directly by gangliosides. These include a ganglioside-stimulated protein kinase and a ganglioside-inhibited protein kinase. Physicochemical studies of these two enzymes revealed that they are distinct from other known protein phosphotransferases such as cAMP-dependent, Ca^{2+} /calmodulin-dependent, and Ca^{2+} /phospholipid-dependent protein kinases. (4) Using a biotinylated probe and sections prepared for both light and electron microscopy, P₀ messenger RNA has been localized in regions of Schwann cell cytoplasm enriched in granular endoplasmic reticulum and along margins of developing myelin sheaths after *in situ* hybridization. The following summary describes these discoveries and the most significant findings obtained in FY 1987 LENP projects.

Cellular Neuropathology Section (CN)

1. Herpes Simplex Virus Type 2 (HSV-2) Pathogenesis and Demyelination.

This project has three aims: (i) To define disease produced by HSV-2 in experimental animal models, including primary and reactivated infections, (ii) to relate neurological disease, particularly CNS demyelination, to factors that influence the outcome of disease, including host age, virus dose and inoculation route, immunological factors, and other host and virus genetics, and (iii) to use insights gained from these models to refine and test a working hypothesis that HSV-2 may have an etiological role in multiple sclerosis (MS).

Published background studies from this laboratory include: (i) The first evidence that HSV-2 can produce multifocal CNS demyelination in mice; demyelination thus produced mimics certain features of MS pathology. One HSV-2 strain we used was initially isolated from the midbrain of an MS patient. (ii) Evidence that HSV-2 can produce non-fatal CNS demyelination by a natural genital route of infection. (iii) Evidence that

thymic cortical necrosis and virus presence in lymphoid tissues segregate with severe CNS disease in acute infections, suggesting that immunosuppression may be induced as a result of HSV-2 infection. (iv) Evidence that immunosuppressive treatment can result in reactivation of latent HSV-2 infection. (v) Evidence suggesting that tract-associated demyelinating lesions arise from a minimal neuronal infection with axonal transport, axon-to-glial spread, and amplification of infection in white matter. (vi) A comparison of epidemiologies of MS and of HSV infections, which suggests that MS could be a low-frequency complication of HSV-2 infection in persons lacking previous protective HSV-1 immunity. These studies are important because they address major plausibility issues which any specific etiological hypothesis for MS must satisfy.

Experiments undertaken, in progress, or completed in FY 1987 attempt to test directly the hypothesis that HSV-2 infection is related to human demyelination disease, and address questions related to the pathogenesis of HSV-2 infection in experimental models.

Studies on human autopsy CNS tissues using immunohistochemical methods show that a few demyelinating lesions contain HSV-2-related antigen. Sections from 31 cases of MS and 34 cases of other neurological diseases (OND) were screened for HSV antigen. In 3/31 MS cases and in one case of brainstem encephalitis, one or more demyelinating lesions contained HSV-2-related antigen. Antigen was detected only with HSV-2 antisera, was restricted to demyelinating lesions and their margins and appeared to be in glial cells. These findings, along with epidemiological and pathogenesis observations, provide strong justification for further studies of the role of HSV-2 in demyelination and in the pathogenesis of MS.

In animal models, experiments show that latent HSV-2 infections may be reactivated by means of immunosuppressive treatment. In animals initially infected by a genital route, the reactivated infection is characterized by antigen presence chiefly in dorsal root ganglia, but also in axons and endoneurial cells of nerves distal to ganglia and, rarely, in motor neurons of the lower spinal cord. Preliminary evidence in subsequent experiments suggest that alternate strategies to infect animals may produce more extensive evidence of reactivated CNS infection.

In another study, early events in primary genital HSV-2 infection were examined, with evidence of peripheral nervous system infection and disease during the first week after virus inoculation. In this study, a method was used that permitted examination of the same region of Epon-embedded tissues by both antigen and ultrastructural methods.

Other studies in progress include: (i) analysis of MS and optic neuritis serum and/or cerebrospinal fluid for HSV antibodies, (ii) examination of mouse and human tissue using in situ hybridization methods to detect HSV genome, (iii) further studies in animal models of reactivated infection in the CNS, and (iv) further examination of human tissues for evidence of HSV antigen.

2. P₀ Glycoprotein Messenger RNA and Expression of P₀ in Developing Peripheral Nervous Tissue.

In situ nucleic acid hybridization techniques offer important new approaches for studies of how synthesis of cellular constituents is regulated in normal tissue and what changes are associated with lesions found in human and experimental diseases. We have used a biotinylated P₀ cDNA to probe sections of developing rat trigeminal nerves which then were subsequently examined by both light and electron microscopy. P₀ messenger RNA was detected in cytoplasmic regions of myelin-forming Schwann cells that were

enriched in granular endoplasmic reticulum. Reaction product localizing P₀ mRNA also was concentrated along the inner and outer margins of developing myelin sheaths. Our results show that the distribution of P₀ messenger RNA can now be studied at the cellular instead of the tissue level by using a biotinylated cDNA probe instead of the radiolabelled probes used previously. This represents a very substantial improvement in methodology. Observations made with this technique are currently being correlated with light and electron microscopic immunocytochemical studies of P₀ glycoprotein expression during development.

Neurotoxicology Section (NT)

1. Myelin Basic Protein, Molecular Mimicry and Human Demyelinating Diseases

This project has examined the possible roles of JC virus T-antigen in demyelinating disease. These roles include the following: (a) disruption of myelin protein processing, e.g., through competition for enzymes such as protein kinases which carry out essential post-translational modifications of myelin basic protein (MBP). We have demonstrated that an amino acid sequence of MBP shared with T-antigen is a recognition sequence for protein kinase C (K.-F. J. Chan, et al. [1986] *Biochem. Biophys. Res. Commun.*); (b) immune-mediated demyelination based on molecular mimicry of myelin proteins by viral antigens. This could involve the same shared sequence which serves as a protein kinase recognition site (triprolyl sequence). Our evidence suggests that the triproline regions of MBP and T-antigen will be cross-reacting (G.L. Stoner, et al. [1986] *Immunol. Invest.*); (c) immune-mediated demyelination involving an attack on T-antigen displayed on the surface of cells. This could be a direct attack on myelin and the oligodendrocyte, or a "bystander effect" following an attack directed toward T-antigen displayed on the surface of astrocytes. Finally, (d) JCV-induced damage to myelin can follow lytic infection of oligodendrocytes in severely immunocompromised patients as in progressive multifocal leukoencephalopathy (PML). Only this latter mechanism is well established. All mechanisms except (b) require the presence of JCV in the brain. Accordingly, we have focused on detection of T-antigen in PML tissue to determine whether T-antigen may be expressed in the absence of capsid synthesis and virion assembly. For this we have developed a double-label immunocytochemical method using a monoclonal antibody to T-antigen and a rabbit antibody to capsid antigens.

2. Gangliosides and Protein Phosphorylation: Roles in CNS Function, Growth, Differentiation and Neurotoxicity.

This project is aimed at determining whether there is a direct relationship between the modulation of gangliosides and protein phosphorylation on neural activities. Gangliosides, a class of sialic acid-containing glycosphingolipids, are major constituents of the membrane components in the CNS and have been implicated to play an important role in certain neuronal functions such as neurotransmission, neurite outgrowth, synaptogenesis, neuronal regeneration, differentiation and development. The exact mode of action of these glycolipids, however, is unknown. Current research in this laboratory attempts to define a molecular basis through which gangliosides may mediate their effects. We found that gangliosides have profound modulatory effects on the phosphorylation of specific proteins in both synaptosomes and myelin. Because post-translational modification of proteins and peptides through phosphorylation and dephosphorylation is of paramount importance in the regulation of numerous biological processes, it seems likely that gangliosides may act as one of the mediators for this mechanism. Two novel protein kinases which can be regulated directly by gangliosides have been discovered.

These include a ganglioside-stimulated protein kinase and a ganglioside-inhibited protein kinase. Physicochemical studies of these two enzymes revealed that they are distinct from the other major protein phosphotransferases such as cAMP-dependent, Ca^{2+} /calmodulin-dependent, and Ca^{2+} /phospholipid-dependent protein kinases. The physiological significance of the ganglioside-modulated protein phosphorylation systems and the functional roles of the ganglioside-stimulated or the ganglioside-inhibited protein kinase in the nervous system have not been established and are subjects of current research. Gangliosides also can regulate the activity of protein kinase C, a pivotal enzyme involved in the regulation of numerous biological processes, at least in vitro. Thus, it is possible that perturbation of the membrane structure which results in changes in the contents or microenvironment of gangliosides may produce a synchronistic action on at least three different protein phosphorylation systems. And as a consequence, various physiological responses can be attained.

The state of phosphorylation of MBP in CNS myelin is highly regulated, albeit the functional significance of this covalent modification and its relationship to myelin formation or demyelinating diseases are yet unknown. Studies in this laboratory show that gangliosides can modulate the extent of phosphorylation of MBP. In addition, Staphylococcus aureus α -toxin, a neurotoxin which has previously been shown to cause demyelination in vivo, also can cause a stimulation of the phosphorylation of this protein in purified myelin. Thus, it seems likely that post-translational modification of myelin basic protein through phosphorylation may play an important role in its interaction with the multilamellar membranes. A novel fragmentation procedure which can generate three major domains of both the phosphorylated and non-phosphorylated forms of MBP has been developed. This method should facilitate future investigations on the structure-function relationship of this protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02549-06 LENP															
PERIOD COVERED October 1, 1986 through September 30, 1987																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Herpes Simplex Virus Type 2 Infection, CNS Demyelination and Multiple Sclerosis																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">J. R. Martin, M.D.</td> <td style="width: 20%;">Medical Officer</td> <td style="width: 15%;">LENP</td> <td style="width: 15%;">NINCDS</td> </tr> <tr> <td>Others:</td> <td>S. Suzuki, M.D.</td> <td>Visiting Fellow</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>H. Webster, M.D.</td> <td>Chief</td> <td>LENP</td> <td>NINCDS</td> </tr> </table>			PI:	J. R. Martin, M.D.	Medical Officer	LENP	NINCDS	Others:	S. Suzuki, M.D.	Visiting Fellow	LENP	NINCDS		H. Webster, M.D.	Chief	LENP	NINCDS
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Others:	S. Suzuki, M.D.	Visiting Fellow	LENP	NINCDS													
	H. Webster, M.D.	Chief	LENP	NINCDS													
COOPERATING UNITS (if any)																	
LAB/BRANCH Laboratory of Experimental Neuropathology																	
SECTION Section on Experimental Neuropathology																	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS: 3.8	PROFESSIONAL: 2.0	OTHER: 1.8															
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project seeks to define the spectrum of acute and recurrent CNS and peripheral disease, including CNS demyelination, which is produced in experimental models of <u>herpes simplex virus type 2 (HSV-2) infection</u>, and to refine and test a hypothesis which relates HSV-2 infection to human <u>demyelinative disease</u>, including <u>multiple sclerosis (MS)</u>. Our previous studies suggest that several major features of HSV epidemiology and pathology are consistent with a possible etiological role for this agent in MS. During FY 1987, studies published or submitted provide evidence that : (i) HSV-2 or related antigen may occasionally be found in human demyelinating lesions, and (ii) that HSV-2 antigen may occasionally be found in the CNS of animals with spontaneous or immunosuppression-induced reactivated infections. Specifically, these studies show that: 1) In 3/31 MS cases and 1/1 case of brainstem encephalitis examined, 1 or more demyelinating lesions contained apparently specific, HSV-2-related antigen. This antigen was found only in tissue sections treated with HSV-2 antisera, and was not found in tissues from other cases with non-demyelinating lesions or in many other demyelinating MS lesions examined. 2) In experimental models, reactivations of HSV-2 infection may occur spontaneously, or be induced by immunosuppression. In a rabbit, spontaneous reactivation occurred, with viral antigen presence and demyelination in spinal cord and brainstem regions. In a mouse model, latent infections established by a genital route were reactivated using high-dose immunosuppression. Viral antigen was chiefly present in neurons in 2 distinct groups of dorsal root ganglia at T9-L2, and L6-S2 levels, but was also found in nerves distal to ganglia, and in occasional neurons in the lower spinal cord.																	

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01995-15 LENP
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Morphological Studies of Myelin Formation, Breakdown and Regeneration		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: H. deF. Webster, M.D. Chief LENP NINCDS Others: L. Lamperth, M.D. Visiting Fellow LENP NINCDS R. Plishka, Ph.D. Biologist LENP NINCDS L. Manuelidis, M.D. Assoc. Prof. Yale University M. Graeber, M.D. Scientist Max Planck Institute		
COOPERATING UNITS (if any) Neuropathology Section, Department of Neurosurgery, Yale Medical School, New Haven, CT; Max Planck Institute for Psychiatry, Munich, Fed. Rep. Germany		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Section on Cellular Neuropathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 2.8	PROFESSIONAL 2.2	OTHER 0.6
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The long range goal of this project is to combine <u>in situ</u> nucleic acid hybridization techniques and immunocytochemical methods to study cellular mechanisms of myelin formation, breakdown and regeneration. Current studies and major findings are: (1) <u>Developmental study of mRNA encoding for P₀ glycoprotein in postnatal rat peripheral nervous tissue by in situ hybridization.</u> Aldehyde-fixed light microscopic vibratome and semithin sections of trigeminal nerves and ganglia from 2d, 7d, 15d, 30d and adult rats were treated with biotinylated P ₀ cDNA according to procedures described by Manuelidis (1985) and Webster et al., (1987). Reaction product that localized P ₀ mRNA was first detected by light and electron microscopy at 2d in cytoplasmic regions of a few myelin-forming Schwann cells that were enriched in granular endoplasmic reticulum. It also was found along the inner and outer margins of developing myelin sheaths. The number of cells containing reaction product and the amount per myelin-forming Schwann cell was highest at 7d and 15d, was less at 30d and still less (but detectable) in the adult. These results are consistent with those obtained previously by Northern blot analysis (Lemke and Axel, 1985). Light and electron microscopic studies that will define the spatial distribution of P ₀ mRNA in Schwann cells during myelin sheath development are in progress. (2) <u>Intracellular distribution of P₀ glycoprotein in developing Schwann cells determined by electron microscopic immunogold staining.</u> A method described by Graeber and Kreutzberg (1985) has been modified to preserve the fine structure of myelin-forming Schwann cells in LR White and Lowicryl-embedded developing trigeminal nerves. Thin sections have been immunostained with a polyclonal antiserum and two monoclonal antibodies. In electron micrographs, anti-P ₀ immunoreactivity was present on developing myelin lamellae and in some cytoplasmic regions of myelin-forming Schwann cells that were enriched in Golgi membranes. Further observations are in progress.		
6/LENP/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02550-06 LENP																				
PERIOD COVERED October 1, 1986 through September 30, 1987																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Immunologic Mechanisms in Virally-induced CNS Demyelination																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: G.L. Stoner, Ph.D.</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 15%;">LENP</td> <td style="width: 15%;">NINCDS</td> </tr> <tr> <td>Others: H. deF. Webster, M.D.</td> <td>Chief, LENP</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>K.-F.J. Chan, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>S.J. Morris, Ph.D.</td> <td>Guest Researcher</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>C.F. Ryschkewitsch, B.S.</td> <td>Medical Technologist</td> <td>LENP</td> <td>NINCDS</td> </tr> </table>			PI: G.L. Stoner, Ph.D.	Senior Staff Fellow	LENP	NINCDS	Others: H. deF. Webster, M.D.	Chief, LENP	LENP	NINCDS	K.-F.J. Chan, Ph.D.	Senior Staff Fellow	LENP	NINCDS	S.J. Morris, Ph.D.	Guest Researcher	LENP	NINCDS	C.F. Ryschkewitsch, B.S.	Medical Technologist	LENP	NINCDS
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Others: H. deF. Webster, M.D.	Chief, LENP	LENP	NINCDS																			
K.-F.J. Chan, Ph.D.	Senior Staff Fellow	LENP	NINCDS																			
S.J. Morris, Ph.D.	Guest Researcher	LENP	NINCDS																			
C.F. Ryschkewitsch, B.S.	Medical Technologist	LENP	NINCDS																			
COOPERATING UNITS (if any) Dept. of Medical Microbiology, Univ. of Wisconsin Med. School, Madison (D.L. Walker); Dept. of Chemistry, Rhodes College, Memphis, TN (R.D. Gilliom)																						
LAB/BRANCH Laboratory of Experimental Neuropathology																						
SECTION Neurotoxicology Section																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																						
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0																				
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has examined the possible roles of JC virus T-antigen in <u>demyelinating disease</u>. These roles include the following: (a) disruption of myelin protein processing, e.g., through competition for enzymes such as protein kinases which carry out essential post-translational modifications of <u>myelin basic protein</u> (MBP). We have demonstrated that an amino acid sequence of MBP shared with <u>T-antigen</u> is a recognition sequence for protein kinase C (K.-F.J. Chan, et al. [1986] Biochem. Biophys. Res. Commun.); (b) immune-mediated demyelination based on molecular mimicry of myelin proteins by viral antigens. This could involve the same shared sequence which serves as a protein kinase recognition site (triprolyl sequence). Our evidence suggests that the triproline regions of MBP and T-antigen will be cross-reacting (G.L. Stoner, et al. [1986] Immunol. Invest.); and (c) immune-mediated demyelination involving an attack on T-antigen displayed on the surface of cells. This could be a direct attack on myelin and the oligodendrocyte, or a "bystander effect" following an attack directed toward T-antigen displayed on the surface of astrocytes. Finally, (d) JCV-induced damage to myelin can follow lytic infection of oligodendrocytes in severely immunocompromised patients as in <u>progressive multifocal leukoencephalopathy</u> (PML). Only this latter mechanism is well established. All mechanisms except (b) require the presence of JCV in the brain. Accordingly, we have focused on detection of T-antigen in PML tissue to determine whether T-antigen may be expressed in the absence of capsid synthesis and virion assembly. For this we have developed a double-label immunocytochemical method using a monoclonal antibody to T-antigen and a rabbit antibody to capsid antigens.																						

7/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02451-07 LENP																				
PERIOD COVERED October 1, 1986 through September 30, 1987*																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular and Molecular Approaches to Neurotoxicology																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">S.J. Morris, Ph.D.</td> <td style="width: 20%;">Guest Worker</td> <td style="width: 10%;">LENP</td> <td style="width: 10%;">NINCDS</td> </tr> <tr> <td>Others:</td> <td>D. Bradley, B.S.</td> <td>Chemist</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>G.L. Stoner, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>A.T. Campagnoni, Ph.D.</td> <td>Professor</td> <td>UCLA Medical School</td> <td></td> </tr> </table>			PI:	S.J. Morris, Ph.D.	Guest Worker	LENP	NINCDS	Others:	D. Bradley, B.S.	Chemist	LENP	NINCDS		G.L. Stoner, Ph.D.	Senior Staff Fellow	LENP	NINCDS		A.T. Campagnoni, Ph.D.	Professor	UCLA Medical School	
PI:	S.J. Morris, Ph.D.	Guest Worker	LENP	NINCDS																		
Others:	D. Bradley, B.S.	Chemist	LENP	NINCDS																		
	G.L. Stoner, Ph.D.	Senior Staff Fellow	LENP	NINCDS																		
	A.T. Campagnoni, Ph.D.	Professor	UCLA Medical School																			
COOPERATING UNITS (if any) Mental Retardation Research Center, UCLA Center for the Health Sciences, Los Angeles, CA 90024																						
LAB/BRANCH Laboratory of Experimental Neuropathology																						
SECTION Neurotoxicology Section																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																						
TOTAL MAN-YEARS 0.0	PROFESSIONAL: 0.0	OTHER: 0.0																				
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) *This project has been discontinued due to the departure of the Principal Investigator. <u>Publication:</u> . S.J. Morris, D. Bradley, A.T. Campagnoni, and G.L. Stoner: Myelin Basic Protein Binds Heme at a Specific Site near the Tryptophan Residue, <u>Biochemistry</u>, 26:2175, 1987.																						

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02699-02

LENP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Roles of Gangliosides in Neuronal and Myelin Function, Cell Growth and Differentiation, and Neurotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K.-F.J. Chan, Ph.D. Senior Staff Fellow LENP NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.6

PROFESSIONAL

1.6

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gangliosides, a class of sialic acid-containing glycosphingolipids ubiquitous in eukaryotic cells, have very profound effects on the phosphorylation of several specific proteins in both synaptosomes and myelin. Two novel protein kinases, a ganglioside-stimulated protein kinase and a ganglioside-inhibited protein kinase, have been identified and partially purified from the particulate fractions of guinea pig brain. These two kinases are distinct from the other major protein phosphotransferases such as cAMP-dependent, Ca^{2+} /calmodulin-dependent and Ca^{2+} /phospholipid-dependent protein kinases. The physiological significance of the ganglioside-stimulated and the ganglioside-inhibited protein kinases is currently under investigation. Gangliosides also can further modulate the activity of protein kinase C, provided phosphatidylserine is present. Thus, our results suggest that gangliosides may exert a synchronistic action on at least three protein phosphorylation systems in brain and may provide more insights into the biochemical mechanisms of the regulation of myelin and neuronal functions by glycosphingolipids. *Staphylococcus aureus* α -toxin, when administered intracerebrally, can cause demyelination of the central nervous system. This toxin has been shown to affect the states of phosphorylation of several proteins in myelin including myelin basic protein. Studies on the direct casual relationship between protein phosphorylation and demyelination are being pursued. The possible functional role(s) of myelin basic protein also have been investigated. A novel fragmentation method has been devised which could trisect this myelin protein into three major domains. Further investigation on the properties of each of these domains involved in the interaction of the multilamellar membranes should generate new understanding on the molecular mechanism of myelination or demyelination.

9/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02525-06 LENP																												
PERIOD COVERED October 1, 1986 through September 30, 1987*																														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Exocytosis Modelling: Kinetics of Membrane Aggregation and Fusion																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: S.J. Morris, Ph.D.</td> <td style="width: 33%;">Guest Worker</td> <td style="width: 33%;">LENP</td> <td style="width: 33%;">NINCDS</td> </tr> <tr> <td>Others: P.D. Smith, Ph.D.</td> <td>Visiting Scientist</td> <td>BEIB</td> <td>DRS</td> </tr> <tr> <td>C.C. Gibson, B.E.</td> <td>Electronics Engineer</td> <td>BEIB</td> <td>DRS</td> </tr> <tr> <td>D. Bradley, B.S.</td> <td>Chemist</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>R. Blumenthal, Ph.D.</td> <td>Section Chief</td> <td>LTB</td> <td>NCI</td> </tr> <tr> <td>A. Walter, Ph.D.</td> <td>Staff Fellow</td> <td>LTB</td> <td>NCI</td> </tr> <tr> <td>D.L. Siegel, Ph.D.</td> <td>Guest Worker</td> <td colspan="2">Proctor and Gamble, Cincinnati, OH</td> </tr> </table>			PI: S.J. Morris, Ph.D.	Guest Worker	LENP	NINCDS	Others: P.D. Smith, Ph.D.	Visiting Scientist	BEIB	DRS	C.C. Gibson, B.E.	Electronics Engineer	BEIB	DRS	D. Bradley, B.S.	Chemist	LENP	NINCDS	R. Blumenthal, Ph.D.	Section Chief	LTB	NCI	A. Walter, Ph.D.	Staff Fellow	LTB	NCI	D.L. Siegel, Ph.D.	Guest Worker	Proctor and Gamble, Cincinnati, OH	
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COOPERATING UNITS (if any) Proctor and Gamble, Inc., Cincinnati, OH																														
LAB/BRANCH Laboratory of Experimental Neuropathology																														
SECTION Neurotoxicology Section																														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																														
TOTAL MAN-YEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0																												
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) *This project has been discontinued due to the departure of the Principal Investigator.																														

10/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02264-11 LENP																				
PERIOD COVERED October 1, 1986 through September 30, 1987*																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models of Neurological Disease																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: S.M. Anderson, Ph.D.</td> <td style="width: 20%;">Guest Worker</td> <td style="width: 20%;">LENP</td> <td style="width: 20%;">NINCDS</td> </tr> <tr> <td>Others: R. Weir, M.D.</td> <td>Guest Worker</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>J.W. Daly, Ph.D.</td> <td>Chief</td> <td>LBC</td> <td>NIADDK</td> </tr> <tr> <td>C.T. Hansen, Ph.D.</td> <td>Animal Geneticist</td> <td>AGRC</td> <td>DRS</td> </tr> <tr> <td>J.T. Petras, Ph.D.</td> <td>Neuroanatomist</td> <td>DNP</td> <td>WRAIR/WRAMC</td> </tr> </table>			PI: S.M. Anderson, Ph.D.	Guest Worker	LENP	NINCDS	Others: R. Weir, M.D.	Guest Worker	LENP	NINCDS	J.W. Daly, Ph.D.	Chief	LBC	NIADDK	C.T. Hansen, Ph.D.	Animal Geneticist	AGRC	DRS	J.T. Petras, Ph.D.	Neuroanatomist	DNP	WRAIR/WRAMC
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J.T. Petras, Ph.D.	Neuroanatomist	DNP	WRAIR/WRAMC																			
COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADDK; Animal Genetic Resource, Division of Research Services; Neuroanatomy Branch, Walter Reed Army Institute of Research, Walter Reed Army Medical Center																						
LAB/BRANCH Laboratory of Experimental Neuropathology																						
SECTION Neurotoxicology Section																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																						
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) *This project has been discontinued due to the departure of the Principal Investigator.																						

11/LENP/DIR

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Molecular Biology
National Institute of Neurological and Communicative
Disorders and Stroke

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Cellular Responses to Hormones and Neurotransmitters Z01 NS 02365-09 LMB	5
Nucleotide Sequence and Control of the <i>gdh</i> Operon Z01 NS 02680-03 LMB	6
Control of Meiosis and Morphogenesis Z01 NS 01886-17 LMB	7

October 1, 1986 through September 30, 1987
Laboratory of Molecular Biology
National Institute of Neurological and Communicative
Disorders and Stroke

Ernst Freese, Ph.D., Chief

Most of the work of the Laboratory of Molecular Biology (LMB) is concerned with the properties and differentiation of mammalian brain cells and the interaction between neurons and astrocytes. cDNAs of genes involved in the synthesis and degradation of excitatory and inhibitory amino acids have been isolated and used to study the appearance of mRNA during development as well as the changes during diseases such as hepatic encephalopathy. It was shown that glutamate binding to neuronal receptors causes the production of cGMP in a Ca-dependent manner and, in the absence of energy sources, effects killing of neurons. Similar to previous observations found in bacteria and yeast, guanine deprivation causes differentiation also in glioma cell lines as demonstrated by the induction of glial fibrillary acidic protein (GFAP). In the attempt to study the interaction between astrocytes and neurons, an astrocyte produced laminin-like protein has been identified as a nerve growth promoting factor and a membrane-associated protein found in both neurons and astrocytes, was found to be identical (or very similar) to a neuronal growth cone protein (GAP-43). The only continuation of microbial studies in LMB concerned the developmental control for glucose dehydrogenase synthesis.

1. Isolation and sequencing of cDNA of genes involved in the metabolism of glutamate and GABA, the expression of these genes in different cell types and its changes during hormonal induction and disease. a) Using antibodies against glutamate dehydrogenase (GDH), cDNA clones have been isolated from brain and liver libraries. When they were sequenced no essential difference was found although the published amino acids sequence obtained from liver GDH has 14 amino acids that differ from the amino acid sequence translated from the cDNA nucleotide sequence. Therefore, the published amino acid sequence apparently is partially incorrect. b) A cDNA of glutaminase has similarly been isolated. Since the corresponding amino acid sequence has never been determined, several methods were used to identify it as genuine glutaminase cDNA: e.g., immunoreactive peptides produced by a lysogen containing the glutaminase cDNA comigrated with those derived from purified brain glutaminase; antibodies purified by affinity to the bacterial protein derived from the cDNA reacted with the subunits of glutaminase. A single glutaminase gene was found in DNA blots. c) Using a genomic clone to hamster glutamine synthetase (GS) a rat brain cDNA clone has been isolated.

Using these and other cDNA clones, it has been shown that the genes for GS and GDH are mostly expressed in astrocytes whereas the genes for glutaminase (GA) and glutamate decarboxylase (GAD) are mostly expressed in neurons. This agrees with the expected functional roles of the corresponding enzymes and their products: glutamine is synthesized in astrocytes, carried over to neurons and converted there to glutamate and, depending on the neuron, further to GABA; these products are, after they have been released as neurotransmitters, taken up again by astrocytes and reconverted to glutamine or degraded. The

developmental changes of the expression of these genes is now being investigated.

Whereas the amount of GS and its mRNA increased in response to dexamethasone addition to cells, the amount of mRNA for the other three genes was not changed. However, these genes are also controlled as was shown by changes in their expression in the brains of rats whose liver was poisoned by thioacetamide. As one might expect from the increased ammonia in the brain, GS message increased, presumably because of increase in the GS enzymes could more rapidly detoxify the poisonous ammonia. The increase in GA mRNA is less immediately obvious but may relate to the fact that ammonia inhibits the activity of GA so that more of the enzyme has to be made in order to produce enough glutamate in neurons. Whereas the GAD message did not change, that of GDH decreased. This agrees with a degradative role of GDH because the decrease of this enzyme would assure that the astrocytes are not too much deprived of glutamate which in the presence of ammonia is rapidly converted to glutamine. It seems likely that hepatic encephalopathy results from hyperammonemia in the brain; interestingly, the brain can protect itself to a limited degree against small increases of ammonia.

2. Membrane transduction and neuronal killing in response to excitatory amino acids. When glutamate binds to granule cells, cultured from the cerebellum of neonatal rat, it stimulates the synthesis of cyclic GMP. The cGMP response can be attributed to the influx of Ca^{2+} which occurs when glutamate binds to the NMDA-type receptor. cGMP is also synthesized by a membrane-bound guanylate cyclase in response to the binding of atrial natriuretic factor (ANF) to cerebellar neurons. This is the first demonstration of the existence of functional ANF receptors on neurons and suggests that ANF may play a role in the management of brain edema.

While some amino acids such as kainic acid are always toxic to neurons, glutamate normally is not. However, when neurons in culture are deprived of glucose, or their ATP production is otherwise prevented, the cells become sensitive to glutamate killing. The reason apparently is that the NMDA-receptor channel is normally blocked by Mg^{++} in a voltage-dependent manner which requires energy for its maintenance. Glutamate also becomes toxic to cerebellar granule cells when magnesium ions are removed from the incubation medium. The toxicity of glutamate is blocked by antagonists of the NMDA-receptor. The mechanism of cell killing is now under investigation.

3. Induction of glial fibrillary acidic protein (GFAP) by deprivation of guanine nucleotides. GFAP is an intermediate filament protein which is made only in astrocytes and can be used as a typical astrocyte marker. However, the immunofluorescence of fixed cells is not a reliable index of the presence of GFAP because many cell lines that seem to be negative in this technique produce the same amount of GFAP as other cell lines when examined by immunoblots. Apparently, GFAP becomes detectable by immunofluorescence only when some GFAP assembly factor is also present.

On prolonged culturing of an astrocytoma cell line, cells were found that had lost the production of GFAP or its RNA. In subclones of such cells the GFAP production was inducible by a small concentration (5 mM) of mycophenolic acid, an inhibitor of IMP dehydrogenase. The effect was counteracted by the addition of guanosine which shows that the deficiency of guanine nucleotides was

specifically responsible for it. These findings demonstrate that cells, which have lost the ability to produce a particular differentiation property, can be induced to a more highly differentiated state by specific deprivation of guanine nucleotides.

4. Properties of brain cell surface proteins such as the neuronal growth cone protein (GAP-43) and a nerve growth promoting factor located in the extracellular matrix of astrocytes. Using two-dimensional peptide mapping of plasma membrane proteins of brain cells, two abundant proteins were observed. One of these proteins was identified as the neuronal growth cone protein GAP-43 by various techniques, including *in vitro* phosphorylation by protein kinase C and reactivity with two antibodies. Interestingly, this protein was present also in rat cortical astrocytes and could be localized by immunofluorescence. The protein is glycosylated, can be labeled by radioiodination of intact astrocytes and therefore apparently reaches through the cell surface. The possibility that this protein is used in the interaction between astrocytes and neurons is now being investigated.

Extracellular matrix material of astrocytes was left on plates when the growing cells were removed by a mild detergent. When PC12 cells were plated on such a surface, they exhibited the outgrowth of long neurites. Application of anti-laminin to this extracellular matrix material resulted in a 60% inhibition of the neurite outgrowth which shows that laminin, a neurite growth promoting factor for peripheral neurons, is also produced by astrocytes. Further experiments are planned to determine whether this astrocyte-specific laminin differs from the laminin found in other cells of the body and to identify the other growth promoting factor present in the extracellular matrix. It was also found that astrocytes cultured for 12 weeks had 2x more neurite-promoting activity than younger ones; this demonstrates that the capability to promote neurite outgrowth is not lost during aging.

5. Developmental control of the gene for glucose dehydrogenase. The mechanism whereby the synthesis of certain enzymes is switched on only during differentiation is still unknown. One such developmental enzyme is glucose dehydrogenase which is made in *Bacillus subtilis* only during forespore development and is located only inside the forespore. The LMB has isolated the gene for this enzyme, found that it is part of an operon, and has sequenced the DNA of the whole operon. Using S₁ nuclease mapping it has determined the location of the transcriptional initiation site to be 12 base pairs from the ribosome binding site that precedes the first gene of the operon. To perform further studies on the control region, the operon was fused to a *lacZ* gene and the gene fusions were inserted into the *B. subtilis* chromosome. Using systematic nuclease deletion mapping, the control region was limited to the area from -35 to 0 nucleotides before the transcription start site. Using *in vitro* mutagenesis, via the incorporation of oligonucleotides, always three bases were changed at a time, because the product of that change was easier to isolate than the change of a single base pair. It became clear that changes in the -35 region and the -10 region were essential whereas some of the area in between these two regions could be modified without any impairment of transcriptional control. Thus, the nucleotides in this region which are important for the control will soon be known and it will then be possible to isolate the control proteins that attach to these essential regions. This will include the RNA polymerase with the necessary sigma-factor and it may include an additional controlling factor which assures that the cell transcribes this gene only during differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02677-03 LMB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Differentiation and Regulation of Gene Expression in Astrocytes and Neurons*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E. Freese, Ph.D.	Chief	LMB, NINCDS
Others:	C. Banner, Ph.D.	Senior Staff Fellow	LMB, NINCDS
	R. Lipsky, Ph.D.	Senior Staff Fellow	LMB, NINCDS
	L. Vitkovic, Ph.D.	Senior Staff Fellow	LMB, NINCDS
	J. Wujek, Ph.D.	Senior Staff Fellow	LMB, NINCDS

COOPERATING UNITS (if any)

Center for Neurochemistry, Strasbourg, France (M. Mersel); Department of Neurology, University of Maryland Medical School (S. Max); Digestive Diseases Branch, NIDDK (K. Mullen)**

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

8

PROFESSIONAL:

7

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigate the regulation of genes encoding proteins important for astrocyte differentiation and the interaction between astrocytes and neurons. We have screened human and rat brain expression libraries for cDNAs encoding glutamine synthetase (GS), glutamate dehydrogenase (GDH) and glutaminase (GA) and determined the nucleotide sequences of the latter two. Dexamethasone induced the synthesis of GS mRNA but not of the other two enzymes. During thioacetamide induced hepatic encephalopathy in the rat, the level of glutamate decarboxylase (GAD) mRNA remained constant, that of GDH decreased whereas the levels of GA and especially GS increased; this agrees with the expectation that glutamate is scarce in astrocytes because it is rapidly converted to glutamine. We have isolated an astrocyte derived cell line which does not produce any glial fibrillary acidic protein (GFAP) or its mRNA; upon GTP deprivation by exposure to mycophenolate, both transcription and transduction are induced about 20- fold.

Some major membrane proteins of brain cells were examined. One was identical or very similar to the major growth cone protein (GAP-43) by MW, isoelectric point, phosphorylation, and antibody staining. We found that this protein is glycosylated and can be labeled with iodine in whole cells, which indicates that it reaches through the membrane to the outside. Since we find the protein also in astrocytes, it may be involved in astrocyte/neuron interaction. We have also identified a neurite growth promoting factor in the extracellular matrix of astrocytes; it may be laminin because the outgrowth is at least partially inhibited by anti-laminin.

* Formerly entitled: Differentiation and Regulation of Gene Expression in Glial Cells

**Cooperating Units Continued:

Epilepsy Branch, NINCDS (S. Nadi); Laboratory of Oto-laryngology, NINCDS (R. Wenthold, M. Schneider); Medical College of Pennsylvania (V. Aloyo); Robert W. Johnson Medical School (W. Nicklas); U. of Arizona, Dept. of Pharmacology (H. Laird); U. of Pittsburgh, Dept. of Biochemistry (N. Curthoys)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02365-09 LMB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Responses to Hormones and Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R. C. Henneberry, Ph.D.	Chief, Molecular Neurobiology Section	LMB, NINCDS
Others:	P. G. Lysko, Ph.D.	Senior Staff Fellow	LMB, NINCDS
	A. Novelli, Ph.D.	Visiting Associate	LMB, NINCDS
	M. D. Collado, Ph.D.	Visiting Fellow	LMB, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Molecular Neurobiology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

4.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our objective is to elucidate how cells derived from the mammalian brain perceive and respond to the signals in their environment. We have studied the effects of hormones and neurotransmitters which transmit information across the plasma membrane while binding to and remaining at cell-surface receptors. In neurons cultured from the cerebellum of neonatal rats, we have shown that the ability to synthesize the second messenger cyclic GMP in response to excitatory amino acids is related to the developmental stage of the neuron, is mediated by the influx of calcium ions, and may be modulated by calmodulin. We have shown that the stimulation of guanylate cyclase by glutamate in cerebellar neurons occurs via the NMDA-subtype of glutamate receptor; when occupied by an appropriate agonist, the receptor opens a sodium-calcium channel which is blocked by magnesium in a voltage-dependent manner. We have now found that the magnesium block is sensitive to the energy level of the cell; decreases in adenine nucleotide levels due to glucose starvation, oxygen deprivation, or metabolic poisons cause sufficient depolarization to relieve the magnesium block of the NMDA receptor channel enabling glutamate to persistently open the channel resulting in excessive influx and cell death. Our results provide experimental evidence for a mechanism which may trigger the transition of glutamate from neurotransmitter to neurotoxin which underlies a variety of neurological disorders.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02680-03 LMB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nucleotide Sequence and Control of the *gdh* Operon*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	K. A. Lampel, Ph.D.	Senior Staff Fellow	LMB, NINCDS
Others:	E. Freese, Ph.D.	Chief	LMB, NINCDS
	Y. Nakatani, Ph.D.	Visiting Fellow	LMB, NINCDS
	K.-D. Neitzke, Ph.D.	Visiting Fellow	LMB, NINCDS

COOPERATING UNITS (if any)

Prof. P. Fortnagel, Inst. für Allgemeine Botanik, West Germany

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.6

PROFESSIONAL:

2.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the developmental process leading to spore production in *Bacillus subtilis*, numerous proteins are synthesized. We have been studying one such protein, glucose dehydrogenase, which is made exclusively during sporulation and only in the forespore compartment. The gene encoding glucose dehydrogenase (*gdh*) is part of a bicistronic unit. From S_1 nuclease mapping experiments, the location of the transcriptional initiation site was determined to be 12 base pairs from the ribosome binding site preceding the first gene of the operon. The region containing the nucleotide sequences needed for *gdh* expression was determined. *Bal*31 nuclease digestions of DNA fragments with the promoter region were fused to a promoterless *lacZ* gene and the gene fusions were inserted into the *B. subtilis* chromosome. Also, by oligonucleotide site directed mutagenesis, alterations in the base sequence at the -35 and -22 region were made. Changes in the -35 region resulted in the loss of *lacZ* expression whereas no effect on *lacZ* expression was noted by change of base sequences in the -22 region. Alterations in the -10 region are currently being assayed. The same technique was employed to make alterations in the *B. subtilis* *gdh* gene near the His 148 and Trp 154 region which is important for subunit binding in the *Bacillus megaterium* glucose dehydrogenase. An insertion of two amino acids (Val, Asn) into the *B. subtilis* *gdh* gene yielded extracts with only 5% of activity as compared to unaltered glucose dehydrogenase.

*Formerly entitled: Nucleotide Sequence Control of the GlcDH Operon

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01886-17 LMB

PERIOD COVERED

October 1, 1986 through September 30, 1987 *

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Meiosis and Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

E. B. Freese, Ph.D.

Biologist

LMB, NINCDS

S. Silverman, Ph.D.

Senior Staff Fellow

LMB, NINCDS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

* This project has been terminated.

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Molecular Genetics

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987
Laboratory of Molecular Genetics
National Institute of Neurological and Communicative
Disorders and Stroke

Robert A. Lazzarini, Ph.D., Chief

Research in the Laboratory of Molecular Genetics includes investigations at both the cellular and molecular level of a number of phenomena that are relevant to medical neurology. In all, there are six interactive programs concerned with the oligodendrocyte and myelin synthesis, viral-host interaction, the developmental biology of the mammalian central nervous system (CNS) and degenerative processes in the CNS.

The myelin sheath is essential to normal conduction in nerves and is altered in multiple sclerosis and Guillain-Barre diseases. Understanding how myelin is formed and repaired requires basic studies on the differentiation of the myelin forming cell, the oligodendrocyte, and on the control of expression of genes encoding the myelin specific proteins. The cell biological and molecular studies of the oligodendrocyte form the linchpin of the laboratory's research efforts. The cell biological studies, headed by Dr. Monique Dubois-Dalq, have employed a unique set of immune reagents to identify and track the early precursors of the oligodendrocyte, called the O-2A progenitor cell. We have found that this cell responds to a mitogen resembling, and perhaps identical to, the platelet derived growth factor (PDGF) which is synthesized by the Type 1 astrocyte. This response to the mitogen can be modulated by tumor growth factor beta (TGF beta). After a number of rounds of cell division driven by the PDGF, the O-2A progenitor evolves into another precursor cell which has acquired new antigens and loses the intermediate filament protein, vimentin, and divides more slowly. These cells can be identified with specific immune reagents using fluorescent microscopy. This intermediate precursor can be stimulated to become a mature oligodendrocyte with insulin-like growth factor 1 (IGF-1) or insulin. This progression of cell types has been observed in oligodendrocytes selected from cell suspensions of brain by cell sorting and also in oligodendrocytes isolated from optic nerves by more classic techniques. The mature oligodendrocyte proceeds to express coordinately the genes encoding the myelin specific proteins. Earlier work by our laboratory has demonstrated that in the absence of neurons these oligodendrocytes are even able to produce whorls of membrane that have a composition very similar to natural myelin.

A question that has plagued neurologists and biologists for years is why the oligodendrocytes of humans do not remyelinate the areas of the brain that have been stripped of their myelin in certain diseases, for example, multiple sclerosis or viral demyelinating diseases. Electron micrographic evidence of others has suggested that the amount of remyelination observed in multiple sclerotic brains is very limited indeed. We are currently analyzing whether CNS remyelination recapitulates the cellular and molecular events of myelination which take place during the normal developmental period. We have established an animal model for demyelination followed by remyelination: mouse hepatitis virus infection of mice leads to demyelinated lesions principally in the spinal cord. After the infection has been cleared by the host's defenses, remyelination of these lesions is quite active. Using *in situ* hybridization and probes for specific myelin genes, we are analyzing the events occurring during this remyelination process. In parallel studies, we are examining the expression of the myelin specific genes around the plaque regions in human multiple sclerotic brain tissue.

Surprisingly, although remyelination is very limited, our in situ hybridization experiments demonstrate that the myelin basic protein (MBP) gene is actively expressed in the vicinity of the demyelinated plaque. This suggests that the first stages of activation of the oligodendrocytes have taken place in the human brain.

During this last reporting period and the previous one, our molecular level studies have focused on aspects of the organization of myelin specific genes: what is the origin of the multiple forms of each of the myelin proteins and what are the features of the myelin specific genes that control their expression and limit it to just the oligodendrocytes and the Schwann cells? To this end, we have cDNA cloned and sequenced both the human and mouse MBP and the proteolipid protein (PLP) mRNAs. We have used these characterized cDNAs to identify and study the organization of the genes encoding them. Our results demonstrate conclusively that the multiple forms of the MBP and PLP found in both man and mouse originate through a mechanism of alternative splicing of the nascent gene transcripts. During the current reporting period, we have completed the initial phases of the characterization of the "promoter" and "enhancer" regions of both the MBP and PLP genes. We have made recombinant plasmid constructions using these genetic elements and are measuring the effect of these elements in transgenic mice. Ultimately, these studies will allow us to place genes under the control of these controller elements and to introduce them into the germ line of mice, thereby, producing mutant mice in which the gene of interest is expressed only in oligodendrocytes or Schwann cells.

Contrary to widely held beliefs, the gene encoding the PLP is expressed in Schwann cells and their tumors. PLP mRNA was identified in human acoustic neuromas and rat and rabbit sciatic nerves using our cDNAs as hybridization probes. The PLP, itself, was shown to be present in rat and human sciatic nerve Schwann cells by immune fluorescent microscopy and by Western blot analyses. Although easily detected in the Schwann cell bodies, the PLP was not detected in the peripheral nervous system (PNS) myelin; therefore, suggesting that PLP is preferentially excluded from this portion of the Schwann cell membrane.

The Laboratory of Molecular Genetics has three programs concerned with viral-host interactions. All involve the study of negative strand RNA viruses, a group of viruses which includes influenza, rabies, measles, mumps, and vesicular stomatitis virus (VSV). In these viruses, the viral RNA synthesis is catalyzed by an RNA polymerase complex which consists of a large viral protein L, a small NS phosphoprotein, and the genomic ribonucleocapsid template. In the first study concerned with the regulation of viral nucleic acid synthesis, Dr. Manfred Schubert and his colleagues have studied the interactions of these proteins in an effort to understand the details of this process. Using recombinant in vitro synthesized NS mutant proteins, purified L protein, and ribonucleocapsids isolated from purified virus, they have identified and mapped two binding domains on opposite halves of the NS protein. The data indicates that the phosphoprotein is a bivalent protein which binds the catalytic subunit L of the polymerase complex at its amino terminal third and the ribonucleocapsid template at its carboxy terminal half. The binding site of the L contains the highly phosphorylated negatively charged region of the protein, while the binding site for the ribonucleocapsid involves a highly conserved carboxy terminal sequence. These studies together with the detailed analysis of the NS protein using monoclonal antibodies have afforded us a more detailed description of the mechanism of RNA synthesis in this important class of viruses.

The replication of the virus and the pathogenesis of a viral disease are influenced by the host genetic factors. In a newly organized research program, Dr. Heinz Arnheiter is studying the biological and molecular characterization of one of these host factors, the Mx system, which specifically protects certain strains of mice against infection with influenza virus. His studies have focused on the structure/function relationship of the Mx protein on the mechanism of the Mx protein

action and on the distribution and function of the Mx system in the animal kingdom. He has shown that a distinct Mx antibody, when microinjected into cells, inhibits the normal accumulation of the mouse Mx protein in the cell nucleus and specifically neutralizes its antiviral activity, rendering the mouse susceptible to influenza virus. Using recombinant DNA techniques, the Mx protein of mice has been expressed in insect cells. We now have a ready source of large amounts of this rare protein which can be used in the study of its mode of action. Experiments are currently underway to examine whether the Mx protein, which is normally expressed in the nucleus of the cell, interferes with that portion of viral replication that normally takes place in the nucleus. To our surprise, it appears even when the Mx protein is present only in the cytoplasm of cells and it confers a resistance to influenza virus.

The assembly of viral particles, the end point of the viral replication process, is a poorly understood phenomenon and only a few techniques exist that can be readily applied to its study. With electron microscopy and immunolabeling techniques for viral proteins, we have determined how the viral nucleocapsid interacts with the viral glycoprotein which is integrated in the plasmid membrane of the infected cell and how the M protein may induce nucleocapsid coiling during viral maturation. Recently, we have extended these studies to include the use of temperature-sensitive mutants of the M protein of VSV and have found that normal transport to the membrane and normal conformation of the M protein are necessary for nucleocapsid coiling and viral budding to occur. Cell fractionation studies and gel analyses show that at nonpermissive temperatures, abnormal M proteins accumulate around the perinuclear membranes. Using deep-etch replica analyses on fast frozen cells, the M protein aggregates of measles virus were visualized for the first time on the inner face of the membrane. These studies have also indicated that abnormal interactions between the measles nucleocapsids and actin filaments may lead to defective budding of the virus.

In our studies of degenerative processes of the nervous system, we have focused our efforts on the characterization of the three subunits of the neurofilaments so that we might be able to assess their contributions, if any, to the neurofibrillary tangles and the amyloid plaques that are found in certain degenerative diseases of the CNS. We have isolated cDNA and genomic clones for the small, the mid-size, and the heavy neurofilament subunits of humans. These cDNA clones were sequenced to determine the amino acid sequence of these proteins, and the genomic clones were studied to define the organization of the genes. Only the amino acid sequence of the small neurofilament subunit of humans was known prior to our studies. We have now completed our sequencing of the mid-size neurofilament subunit and have reported on the complete amino acid sequence of that protein. At present, we have approximately 60% of the heavy neurofilament subunit cDNA characterized and anticipate completing this subunit within a few months. The predicted protein for the mid-size neurofilament subunit contains a serine rich 13 amino acid sequence repeated tandemly six times. This sequence is missing from the small neurofilament, but a related sequence which repeated 50 to 100 times has been identified in the heavy neurofilament subunit. These repeated amino acid sequences occur in a region of the protein which had previously been shown to contain the phosphorylation sites. The small neurofilament which does not contain this repeated sequence is not phosphorylated. Our speculation that this was the site of phosphorylation of the mid and large subunits has been confirmed during this last reporting period. We have synthesized peptides corresponding to the repeat subunit and have chemically phosphorylated them. A large panel of monoclonal antibodies recognizing neurofilaments was assembled and tested for their ability to recognize the various forms of the synthetic peptides. Of the 500 or so monoclonal antibodies tested, 96 recognized one or another form of the synthetic peptides. Those monoclonal antibodies which were completely specific for the phosphorylated form of neurofilaments recognized only the phosphorylated form of the synthetic peptides, proving that this site is, in fact, the major site of phosphorylation for the neurofilaments. Interestingly, the antibodies raised against this phosphorylated synthetic peptide also recognize neurofibrillary tangles

isolated from Alzheimer's brain. It is unclear at present whether this amino acid sequence is a general phosphorylation site found on many proteins or whether it is specific for neurofilaments. Consequently, we do not know if the material in the neurofibrillary tangles recognized by the antibodies is derived from neurofilaments.

Homeo domain proteins have been shown to participate in the development of Drosophila. The significance of the homeo domain proteins has been highlighted by the recent demonstration that homeo domain proteins are also found in other metazoans, including mice and men. These observations suggest the exciting possibility that genes which control Drosophila development may share some partial homology to genes encoding functions important in the development of mammalian organisms. Thus, it has been possible to use Drosophila genes to identify structurally related genes in the mouse genome. By identifying the functional role of these mouse genes, it may be possible to gain insight into the principles which govern mammalian development. In a new program, we are examining the question of whether specific homeo box genes and homeo domain proteins play a role in the development of the mammalian CNS. As an initial step, we have focused our efforts on the molecular biology of the murine Hox 1.3 homeo box gene and its encoded protein. We have analyzed both the cDNA and genomic clones of this gene. The gene is organized into two exons and encodes a 270 amino acid homeo domain protein. mRNA analysis shows that the gene is expressed early in embryogenesis, but that transcripts are also present in many adult tissues. We have prepared antibodies against synthetic peptides corresponding to portions of the protein and have used these in the immune localization of the protein in sections of embryonic and adult tissue. In the adult CNS, certain subsets of fully differentiated neurons express the Hox 1.3 protein. The Purkinje neurons of the cerebellum, the pyramidal and the dentate neurons of the hippocampus, and the motor neurons of the spinal cord display large amounts of this protein. Work is currently underway to examine the function of this protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02034-15 LMG																								
PERIOD COVERED October 1, 1986 through September 30, 1987																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Biology of Myelin-Forming Cells <u>In Vitro</u> and <u>In Vivo</u> Including Remyelination*																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: M. Dubois-Dalcq, M.D.</td> <td style="width: 40%;">Section Chief</td> <td style="width: 20%;">LMG, NINCDS</td> </tr> <tr> <td>Others: V. Friedrich, Ph.D.</td> <td>Special Expert</td> <td>LMG, NINCDS</td> </tr> <tr> <td>C. Jordan, Ph.D.</td> <td>Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>N. Zeller, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>T. Behar, B.S.</td> <td>Microbiologist</td> <td>LMG, NINCDS</td> </tr> <tr> <td>R. Rusten</td> <td>Biological Lab Technician</td> <td>LMG, NINCDS</td> </tr> <tr> <td>C. Godfraind, M.D.</td> <td>Visiting Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>K. Ono, M.D., Ph.D.</td> <td>Visiting Fellow</td> <td>LMG, NINCDS</td> </tr> </table>			PI: M. Dubois-Dalcq, M.D.	Section Chief	LMG, NINCDS	Others: V. Friedrich, Ph.D.	Special Expert	LMG, NINCDS	C. Jordan, Ph.D.	Staff Fellow	LMG, NINCDS	N. Zeller, Ph.D.	Senior Staff Fellow	LMG, NINCDS	T. Behar, B.S.	Microbiologist	LMG, NINCDS	R. Rusten	Biological Lab Technician	LMG, NINCDS	C. Godfraind, M.D.	Visiting Fellow	LMG, NINCDS	K. Ono, M.D., Ph.D.	Visiting Fellow	LMG, NINCDS
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K. Ono, M.D., Ph.D.	Visiting Fellow	LMG, NINCDS																								
COOPERATING UNITS (if any) K.V. Holmes and D. Weismiller, Dept. Pathology, USUHS; L. Hudson, Lab. Molecular Genetics, NINCDS; W. Richardson, Univ. College, London; F. deFerra and F.A. McMorris, Wistar Inst.; E. Schilling, Lab. Chemoprevention, NCI; J. Barker and E. Novotny, Lab. Neurophysiology, NINCDS.																										
LAB/BRANCH Laboratory of Molecular Genetics																										
SECTION Neural and Molecular Ultrastructure Section																										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																										
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CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The myelin sheath is essential to normal conduction in nerves and is altered in multiple sclerosis and Guillain-Barre diseases. Understanding how myelin is formed and repaired requires basic studies of the differentiation of myelin-forming cells both <u>in vitro</u> and <u>in vivo</u>. We are now studying the precursor cells of oligodendrocytes in the developing rat CNS and the molecules that trigger their mitosis and differentiation. We found that the major mitogen for the 0-2A progenitor cells is a PDGF-like molecule synthesized by Type 1 astrocytes and that TGF beta, also synthesized by these cells, may modulate the PDGF effects. After a number of mitoses driven by PDGF, the 0-2A progenitor evolves into another precursor which acquires O₄ antigen, loses vimentin, divides more slowly and can be triggered to become an oligodendrocyte by IGF-1 or insulin. We have also shown that 0-2A progenitors sorted from the brain have properties identical to those characterized in optic nerve, but have higher mitogenic potential. Investigations are being carried out to determine if CNS remyelination recapitulates cellular and molecular events of myelination by using immunocytochemistry on frozen sections combined with tritiated thymidine pulse. Characterization of the dividing precursors of oligodendrocytes around the lesions of animals with active demyelination produced by a corona virus are also being performed. Using <u>in situ</u> hybridization and probes specific for some myelin gene exons, we are examining whether splicing events in remyelination occur as in myelination. Additional studies on myelin deficient rats are aimed at analyzing how differentiation of oligodendrocytes is regulated when expression of one major myelin gene, proteolipid, is altered. Expression of proteolipid was unexpectedly found to occur in Schwann cells although peripheral myelin does not contain this protein.																										
*[Formerly: "Biology of Myelin Forming Cells <u>In Vitro</u> and <u>In Vivo</u> "]																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02528-06 LMG																								
PERIOD COVERED October 1, 1986 through September 30, 1987																										
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Regulation of Myelin Synthesis																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: R. A. Lazzarini, Ph.D. </td> <td style="width: 33%; vertical-align: top;"> Chief </td> <td style="width: 33%; vertical-align: top;"> LMG, NINCDS </td> </tr> <tr> <td colspan="3" style="padding-top: 10px;"> Others: <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">L. Hudson, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LMG, NINCDS</td> </tr> <tr> <td>J. Kamholz, M.D., Ph.D.</td> <td>Medical Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Toffenetti, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Chambers, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>W. Jacob, Ph.D.</td> <td>Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Berndt, B.S.</td> <td>Microbiologist</td> <td>LMG, NINCDS</td> </tr> </table> </td> </tr> </table>			PI: R. A. Lazzarini, Ph.D.	Chief	LMG, NINCDS	Others: <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">L. Hudson, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LMG, NINCDS</td> </tr> <tr> <td>J. Kamholz, M.D., Ph.D.</td> <td>Medical Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Toffenetti, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Chambers, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>W. Jacob, Ph.D.</td> <td>Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Berndt, B.S.</td> <td>Microbiologist</td> <td>LMG, NINCDS</td> </tr> </table>			L. Hudson, Ph.D.	Senior Staff Fellow	LMG, NINCDS	J. Kamholz, M.D., Ph.D.	Medical Staff Fellow	LMG, NINCDS	J. Toffenetti, Ph.D.	Senior Staff Fellow	LMG, NINCDS	J. Chambers, Ph.D.	Senior Staff Fellow	LMG, NINCDS	W. Jacob, Ph.D.	Staff Fellow	LMG, NINCDS	J. Berndt, B.S.	Microbiologist	LMG, NINCDS
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INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																										
TOTAL MAN-YEARS: 5.75	PROFESSIONAL: 4.75	OTHER: 1.0																								
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The objective of this umbrella project is to acquire a detailed understanding of the developmental program that culminates in myelin synthesis. We are particularly interested in obtaining molecular level information about the control of expression of both the myelin basic protein (MBP) and proteolipid protein (PLP) genes. To this end, we have cDNA cloned and sequenced both the human and mouse MBP and PLP mRNAs. We have used these characterized cDNAs to identify and study the organization of the genes encoding them. Our results demonstrate conclusively that the multiple forms of MBP and PLP found both in man and mouse originate through a mechanism of alternative splicing of the nascent gene transcript. During the current reporting period, we have completed the initial phases of the characterization of the promoter and enhancer regions of both the MBP and the PLP genes. We have made plasmid constructions using these <u>cis</u> acting elements and a gene whose expression is easily measured. These constructions have been introduced into the germ line of mice so that the tissue specificity of these <u>cis</u> acting elements can be verified in an <u>in vivo</u> animal model. Contrary to widely held beliefs, the gene encoding proteolipid protein is expressed in Schwann cells and their tumors. PLP mRNA was identified in human acoustic neuromas and rat and rabbit sciatic nerves using PLP cDNA as a probe. The proteolipid protein, itself, was shown to be present in rat and human sciatic nerve Schwann cells by immune fluorescent microscopy and Western blot analysis. Although easily detected in Schwann cell bodies, the PLP was not detected in the peripheral myelin, itself, suggesting that the PLP is preferentially excluded from this portion of the Schwann cell membrane.																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02026-15 LMG									
PERIOD COVERED October 1, 1986 through September 30, 1987											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Viral Nucleic Acid Synthesis in Animal Cells											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: M. Schubert, Ph.D. </td> <td style="width: 33%; vertical-align: top;"> Research Chemist </td> <td style="width: 33%; vertical-align: top;"> LMG, NINCDS </td> </tr> <tr> <td colspan="3" style="padding-top: 10px;"> Others: G. Harmison, II, B.S. P. Brayton, Ph.D. S. Müllner, Ph.D. S. Emerson, Ph.D. </td> </tr> <tr> <td></td> <td style="vertical-align: top;"> Chemist Staff Fellow Guest Researcher Guest Researcher </td> <td style="vertical-align: top;"> LMG, NINCDS LMG, NINCDS LMG, NINCDS LMG, NINCDS </td> </tr> </table>			PI: M. Schubert, Ph.D.	Research Chemist	LMG, NINCDS	Others: G. Harmison, II, B.S. P. Brayton, Ph.D. S. Müllner, Ph.D. S. Emerson, Ph.D.				Chemist Staff Fellow Guest Researcher Guest Researcher	LMG, NINCDS LMG, NINCDS LMG, NINCDS LMG, NINCDS
PI: M. Schubert, Ph.D.	Research Chemist	LMG, NINCDS									
Others: G. Harmison, II, B.S. P. Brayton, Ph.D. S. Müllner, Ph.D. S. Emerson, Ph.D.											
	Chemist Staff Fellow Guest Researcher Guest Researcher	LMG, NINCDS LMG, NINCDS LMG, NINCDS LMG, NINCDS									
COOPERATING UNITS (if any) Dr. Sue Emerson, University of Virginia School of Medicine, Charlottesville, VA; Drs. Monique Dubois-Dalcq and Kenzo Ono, Neural and Molecular Ultrastructure Section, LMG.											
LAB/BRANCH Laboratory of Molecular Genetics											
SECTION Molecular Virology Section											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS 3.63	PROFESSIONAL: 2.58	OTHER 1.05									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> ☐ (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> ☒ (c) Neither </td> </tr> </table>			☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither						
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither									
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Strategies for the prevention or treatment of viral infections focus mainly on two targets: the viral surface proteins or the viral replication system. Our efforts focus on the packaged replicase of nonsegmented negative strand viruses. Little is known on the molecular level about the essential, viral specific mechanisms involved in transcription and replication of these viruses. Our goal is to identify and to characterize in detail viral specific mechanisms which could be specifically inactivated without affecting the host cell. With respect to the study of the RNA dependent RNA polymerase of nonsegmented negative strand viruses such as measles, mumps, rabies, etc., vesicular stomatitis virus has become the prototype virus. The multifunctional VSV RNA polymerase functions as a transcriptase and as a replicase and aberrations of its functions can indirectly affect the cytopathogenicity of the virus. The VSV polymerase complex consists of the large viral protein L, the small NS phosphoprotein, and the genomic ribonucleocapsid template. These proteins are absolutely required for the synthesis of five viral messenger RNAs as well as the genomic RNA during replication. Complete cDNA of these genes, isolated in our lab, are being used for our studies. Using recombinant, <u>in vitro</u> synthesized, NS deletion mutant proteins, purified L, ribonucleocapsids, and specific antibodies, we have identified and mapped two binding domains in opposite halves of the NS protein. This data suggests that the phosphoprotein NS is a bivalent protein which binds the catalytic subunit L of the polymerase complex at its amino terminal third and the ribonucleocapsid template at the carboxy terminal half. The binding site for L contains the constitutively phosphorylated, highly negatively charged region of the NS protein while the binding site for the ribonucleocapsid involves the 21 highly conserved carboxy terminal amino acids.											
7 - LMG/DIR											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02742-01 LMG
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Mechanisms of Viral Pathogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: H. Arnheiter, M.D.	Visiting Scientist	LMG, NINCDS
Others: G. Weitz, Ph.D. L. Richter-Mann	Visiting Fellow Biologist	LMG, NINCDS LMG, NINCDS
COOPERATING UNITS (if any)		
Professor Charles Weissmann, Institute for Molecular Biology, University of Zurich; Professor Otto Haller and Dr. Ellen Meier, Institute for Immunology and Virology, University of Zurich.		
LAB/BRANCH Laboratory of Genetic Mutagenesis		
SECTION Recombinant Genetics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 3	PROFESSIONAL: 3	OTHER 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Pathogenesis and epidemiology of viral diseases, be they neurotropic or not, are influenced by genetic factors specified by both virus and host. Our studies deal with the biological and molecular characterization of one of these host factors, the <u>Mx</u> system, which is operative specifically against influenza viruses. Starting with the original observation that mice of one particular inbred strain spontaneously survive experimental influenza infection, a single gene existing in two allelic forms, <u>Mx+</u> and <u>Mx-</u>, was found to control the course of infection: the presence of <u>Mx+</u> is correlated with influenza resistance, and that of <u>Mx-</u> with influenza susceptibility. In <u>Mx+</u> cells, but not <u>Mx-</u> cells, interferon induces a unique protein, the <u>Mx</u> protein. This protein, when constitutively expressed by recombinant DNA techniques in <u>Mx-</u> cells, confers specific protection against influenza virus infection in culture. Our studies focus on the structure/function relationship of the <u>Mx</u> protein, on the mechanism of <u>Mx</u> protein action, on distribution and function of the <u>Mx</u> system in the animal kingdom, and on the transgenic approach to transfer influenza resistance to <u>Mx-</u> animals. We have shown that a distinct <u>Mx</u> antibody, when microinjected into cells, inhibits the normal accumulation of the mouse <u>Mx</u> protein in the cell nucleus and specifically neutralizes its antiviral activity. A nuclear transport signal on the mouse <u>Mx</u> protein has also been identified and its absence has been shown to reduce, but not abolish, the protein's anti-influenza activity. The mouse <u>Mx</u> protein has been expressed in insect cells and the recombinant protein determined to be active. We have also shown that the <u>Mx</u> cognates of at least one additional species, the rat, act in an influenza specific way much as the mouse <u>Mx</u> protein does. Finally, we have produced four <u>Mx</u> transgenic animals which are currently being analyzed for expression of <u>Mx</u> protein in various tissues and for antiviral resistance <u>in vivo</u>.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02600-05 LMG
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Assembly of Enveloped RNA Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Dubois-Dalcq, M.D.	Section Chief LMG, NINCDS
Others:	C. Jordan, Ph.D.	Staff Fellow LMG, NINCDS
	K. Ono, M.D., Ph.D.	Visiting Fellow LMG, NINCDS
	R. Rusten	Biological Lab Technician LMG, NINCDS
COOPERATING UNITS (if any) Dr. M. Schubert, Molecular Virology Section, Laboratory of Molecular Genetics, NINCDS; and Dr. J. Heuser, Washington University, St. Louis, Missouri.		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Section on Neural and Molecular Ultrastructure		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.75	0.5	0.25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The elucidation of mechanisms of synthesis, processing and transport of viral components at the molecular level will help us to understand problems of defective viral assembly implicated in some neurological diseases. To study the biosynthesis of viral macromolecular components, we prepared monoclonal antibodies reacting with 1) different sites of polypeptides of two negative stranded RNA viruses (vesicular stomatitis virus and measles virus), 2) polyclonal antibodies made against synthetic peptides corresponding in sequence to portions of the viral polypeptides, and 3) genes coding for some of the viral polypeptides either cloned into convenient expression vectors or labeled to detect viral mRNAs in cells by <u>in situ</u> hybridization. With electron microscopy and immunolabeling techniques for viral proteins, we have determined how the nucleocapsid interacts with the viral glycoprotein which is integrated in the plasma membrane and how the M protein may induce nucleocapsid coiling during viral maturation. Studies with a temperature-sensitive mutant in the M protein of VSV indicate that normal transport of the M protein to the membrane and normal conformation are necessary for nucleocapsid coiling and viral budding to occur. Cellular fractionation studies and gel analyses show that, at nonpermissive temperatures, abnormal M proteins accumulate around the perinuclear membranes. Using the deep-etch replica analyses on fast frozen cells, the M protein aggregates of measles were visualized for the first time on the inner face of the membrane. Abnormal interactions between measles nucleocapsids and actin may lead to defective budding.		
9 - LMG/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02714-02 LMG
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.) Degenerative Processes of the Nervous System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	R. A. Lazzarini, Ph.D.	Chief LMG, NINCDS
Others:	P. Shneidman, M.D.	Medical Staff Fellow LMG, NINCDS
	M. Carden, Ph.D.	Guest Researcher LMG, NINCDS
	J. Lees, Ph.D.	Visiting Fellow LMG, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Recombinant Genetics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have isolated cDNA and genomic clones for the small, mid-size, and heavy neurofilament subunits of humans. These cDNA clones were sequenced to determine the amino acid sequence of the protein and the genomic clones were sequenced to define the organization of the genes. The predicted protein of the mid-size neurofilament contains a serine rich 13 amino acid sequence repeated tandemly six times in the carboxy terminus tail region. The sequence is missing from the small neurofilament, but a related sequence has been identified in the carboxy terminal region of the heavy neurofilament subunit. In the latter case, there may be as many as 50 to 100 tandem repeats of this sequence. The fact that these repeats occur in regions of the proteins known to be phosphorylated, prompted us to speculate that they were the site of phosphorylation. The repeated region appears to allow the otherwise helical protein to bend, indicating that phosphorylation at the site might alter the protein's conformation. Our speculations that this was the site of phosphorylation have been confirmed. We have synthesized peptides corresponding to the repeat subunit and a triplication of the repeat subunit. Samples of these peptides were chemically phosphorylated. A large panel of monoclonal antibodies recognizing neurofilaments was assembled and tested for their ability to recognize the various forms of the synthetic peptides. Of the 500 monoclonal antibodies tested, 96 recognized one or another form of the synthetic peptides. Those monoclonal antibodies which were completely specific for the phosphorylated form of neurofilaments recognized only the phosphorylated form of the synthetic peptides, proving that this site is, in fact, the major site of phosphorylation of the neurofilaments. Circular dichroism measurements substantiated our prediction that phosphorylation at these sites dramatically changes the conformation of the protein.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02698-02 LMG																								
PERIOD COVERED October 1, 1986 through September 30, 1987																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Mammalian Homeo Domain Proteins																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: W. Odenwald, Ph.D.</td> <td style="width: 30%;">Microbiologist</td> <td style="width: 30%;">LMG, NINCDS</td> </tr> <tr> <td>Others: C. Taylor, Ph.D.</td> <td>Visiting Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>J. Garbern, M.D., Ph.D.</td> <td>Medical Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td>E. Tournier-Lasserre, M.D.</td> <td>Guest Researcher</td> <td>LMG, NINCDS</td> </tr> <tr> <td>M. Tani</td> <td>Guest Researcher</td> <td>LMG, NINCDS</td> </tr> <tr> <td>V. Friedrich, Ph.D.</td> <td>Special Expert</td> <td>LMG, NINCDS</td> </tr> <tr> <td>K. Farrell</td> <td>Microbiologist</td> <td>LMG, NINCDS</td> </tr> <tr> <td>F. Palmer-Hill, M.S.</td> <td>Geneticist</td> <td>LMG, NINCDS</td> </tr> </table>			PI: W. Odenwald, Ph.D.	Microbiologist	LMG, NINCDS	Others: C. Taylor, Ph.D.	Visiting Fellow	LMG, NINCDS	J. Garbern, M.D., Ph.D.	Medical Staff Fellow	LMG, NINCDS	E. Tournier-Lasserre, M.D.	Guest Researcher	LMG, NINCDS	M. Tani	Guest Researcher	LMG, NINCDS	V. Friedrich, Ph.D.	Special Expert	LMG, NINCDS	K. Farrell	Microbiologist	LMG, NINCDS	F. Palmer-Hill, M.S.	Geneticist	LMG, NINCDS
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Homeosis refers to a class of mutations which brings about the replacement of one body part with that of another normally found elsewhere on the animal. Molecular analysis of the <u>Drosophila</u> homeotic regulatory genes has shown that the proteins which they encode contain a highly conserved 60 amino acid domain (the homeo domain). Homeo domain containing proteins are part of the mechanism which controls the diversity of body parts in <u>Drosophila</u>. The significance of the homeo domain has been highlighted by the recent demonstration that homeo domain proteins are found in other metazoans, including mice and humans. These observations suggest the exciting possibility that genes which control insect body plan may have partial homology to functionally related genes which control the body plans of mammals. By identifying the functional roles these genes play in mouse development, we hope to gain insight into the basic principles which govern mammalian development. As an initial step, we have focused our efforts on the molecular biology of the murine <u>Hox 1.3</u> homeo box gene and its encoded protein. DNA sequence analysis of <u>Hox 1.3</u> cDNA and genomic clones indicates that the gene is organized into two exons and encodes a 270 amino acid homeo domain protein. Messenger RNA analysis shows that the gene is expressed early in embryogenesis. <u>Hox 1.3</u> transcripts are also present in many adult tissues which possess different cell lineages. Immunolocalization studies detect the <u>Hox 1.3</u> protein in many cell types during embryonic development, most notably in the spinal ganglia. In the adult CNS, certain subsets of fully differentiated neurons express the <u>Hox 1.3</u> protein. The Purkinje neurons of the cerebellum, the pyramidal and dentate neurons of the hippocampus, and the motor neurons of the spinal cord are positive.																										

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neural Control, Division of Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987
Laboratory of Neural Control, Division of Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

Research work in the Laboratory of Neural Control (LNLC) is devoted to studies of the central and peripheral neural mechanisms involved in the control of movement in mammals, emphasizing neural organizations at the level of the spinal cord and those regions of the brain stem and cerebral cortex that project directly to the spinal cord.

Present Organization

During FY 1987, the staff of the Laboratory of Neural Control (LNLC) include, 12 professional scientists (four permanent senior scientists and eight post-doctoral fellows). The permanent staff also includes seven full-time permanent support personnel (a physiologist, two engineers, one computer programmer, one biological technician, and one laboratory secretary). Non-permanent staff includes three graduate students, two engineering aides, and one laboratory aide. In FY 1986 two sections were formed: 1) a Section on Neural Mechanisms; and 2) a Section on Neurokinesiology. The research effort in LNLC can be described under four general headings:

1. Electrophysiological and morphological analysis of the cellular physiology and neuronal circuitry operating in the control of movement at the spinal cord level in acute, immobile animals (primarily cats), either anesthetized or unanesthetized following acute destruction of the supratentorial brain (decerebrate preparations).
2. Analyses of motor mechanisms that utilize novel methods for recording the activity of individual neural elements, activity patterns in whole muscles, and kinesiological data in awake, intact animals (both cat and monkey) that are comfortable and performing normal motor behaviors.
3. Theoretical and computer modeling studies of cellular biophysical properties of single neurons, information processing in neural networks, kinesiological properties of moving limbs, and the properties of complex proprioceptive elements such as muscle spindles.
4. Activities concerned directly with the development of new instruments and techniques, and the further refinement of existing methods, for recording and analyzing neurally-relevant data from intact, freely moving animals and for computer-assisted reconstruction of the anatomy of functionally identified neural elements.

Project Summaries:

Many of the projects underway in LNLC are interactive with one another, and staff members often participate in sub-projects that come under separate headings. Points of overlap between projects included under separate headings will be apparent.

Motor Control Systems in the Spinal Cord: This project and the following one utilize cats, either anesthetized or after decerebration, with destruction of the supratentorial brain. The neural mechanisms that control movement are inferred from data obtained in reduced, immobile preparations. When survival surgery is required, as in studies of the morphology of motor nuclei using retrograde transport

methods, surgery is performed under anesthesia and aseptic conditions, and appropriate postoperative care is supplied.

The main goal of these projects is to examine the organization of neuronal systems in the mammalian spinal cord that are involved in the control of movement. Specific topics range from studies of cellular properties of individual neurons in identified neuronal systems to examination of the organization and function of specific neuronal circuits that are involved in movement control, including the problem of central pattern generation at spinal segmental levels.

During FY 1987, we extended our studies of the organization of excitatory interneurons that project directly to motoneurons in the cat spinal cord, with emphasis on the input pathways from distal areas of hindlimb skin. We have shown that cutaneous afferents with low electrical thresholds (less than 2xT) produce excitatory postsynaptic potentials (EPSPs) in certain motor nuclei, particularly in flexor digitorum longus (FDL) alpha-motoneurons, with central latencies consistent with disynaptic connection (less than 1.8 msec). This fact is important because, in principle, interneurons in disynaptic pathways can be identified as individual by virtue of receiving defined, accessible inputs (e.g., particular species of primary afferents of supraspinal descending fiber systems) and by their projection to functionally defined species of neurons, in this case, alpha-motoneurons of a particular motor nucleus. We have shown that the initial excitatory components in cutaneous PSPs in FDL motoneurons are strongly facilitated by stimulation of descending motor systems, including the rubrospinal and pyramidal tracts, and are also markedly facilitated during particular phases of the step cycle that is produced by the spinal central pattern generator (CPG) for locomotion. These patterns may make it possible, for the first time, to isolate and identify individual spinal interneurons that directly excite alpha motoneurons.

Using the spatial facilitation techniques pioneered by Lundberg, we have shown that cutaneous EPSP components produced in FDL motoneurons by electrical stimulation of the cutaneous division of the superficial peroneal nerve (cSP) are markedly enhanced during the flexion phase of fictive stepping. This demonstrates that the spinal CPG, which can be activated by several techniques in immobilized, decerebrate animals (fictive locomotion), also excites interneurons in short latency, excitatory cutaneous pathways. This observation has several interesting correlates in terms of the modulation of corrective reflex actions that must be controlled differentially during the stepping cycle. It also raises the possibility that the excitatory last-order interneurons in the cutaneous reflex pathways may also be utilized by the spinal cord to distribute excitatory drive from the locomotion CPG to alpha-motoneurons. More recently, we showed that EPSPs produced in FDL motoneurons after stimulation of the nerves supplying the central plantar pad (PLn) are enhanced during the extension phase of fictive stepping. In addition, we have found facilitation of cutaneous EPSPs (from cSP, saphenous and sural nerves) in other species of flexor and extensor hindlimb motoneurons during the flexion phase, with specific patterns evident that depend on the cutaneous nerve stimulated and on the motor nucleus being recorded. Although interpretation of the results is complicated by the presence of changing cell input conductance in the recorded motoneurons, particularly when recording from extensor cells, the available evidence suggests that multiple, independent sets of excitatory interneurons are present in spinal segments which convey information from particular regions of the distal hindlimb skin which receive organized convergent control from the segmental CPG.

Considerable further work will be required in order to sort out the patterns of CPG control and their possible roles in modulating locomotor behavior in the intact animal. However, the spatial facilitation results provide important background information for a proposed search for individual interneurons that can: 1) be identified as belonging to particular skin reflex pathways; and 2) that project to particular groups of alpha motoneurons.

We have examined the effect of (-)Baclofen on transmission between group Ia afferents and alpha motoneurons in the cat spinal cord. The drug (-)Baclofen appears to activate GABA-B receptors and produces a decrease in voltage-sensitive Ca^{++} influx, thereby reducing the release of neurotransmitters.

Its effect on group Ia synaptic transmission is to markedly reduce the amplitude of monosynaptic Ia EPSPs in motoneurons and to markedly increase the relative increase in EPSP amplitude following a high frequency tetanus to the same Ia afferents (post-tetanic potentiation, or PTP). This is associated with a decrease, and in some cases abolition, of the post-tetanic depression of Ia EPSPs, which normally occurs in parallel with PTP. The effect of (-)Baclofen thus mimics that of low external Ca^{++} concentration at more accessible synapses. These experiments indicate that GABA-B receptors are present on group Ia synaptic terminals, where they may play a role in the phenomenon of presynaptic inhibition.

Intrinsic Properties of Motor Units: The major focus of this project is to describe in detail the morphological and electrophysiological characteristics of alpha-motoneurons in relation to their motor unit type - i.e., the properties of the muscle fibers innervated by them. We have developed an extensive data base on the detailed morphology of type-identified motoneurons in the triceps surae motor nucleus of the cat, together with the most extensive collection of information on the distribution of group Ia monosynaptic contacts to them that is available to date. These data have been used to estimate the membrane properties of the different motor unit types, and to develop computer models that permit exploration of the factors that control the efficacy of central nervous systems synapses. Our data suggests that the dendritic trees of motoneurons innervating fast versus slow twitch muscle units are qualitatively, as well as quantitatively different from one another. The passive membrane resistivity of these cell types also appears to be different, as are certain details of the organization of synaptic input to them. All these data, when taken together, argue that the alpha-motoneurons that make up a single motor nucleus in fact form at least two distinct subclasses. This conclusion has important implications for theories about the development of the CNS and the specificity of its elements that are under genetic control.

Recent work on characterization of the motor unit population in the cat tenuissimus (TEN) muscle has been undertaken in collaboration with an investigator at the Hebrew University, Jerusalem, Israel. The main aim of the work is two-fold: 1) to provide information on the motor unit population of a muscle with highly unusual internal architecture (staggered serial muscle unit distribution); and 2) to characterize a motor unit system that may be useful to examine in detail the probable difference in neuromuscular junction physiology in different motor unit types. The motor unit characterization studies were completed in FY 1987 and the neuromuscular physiology will now be studied in Jerusalem.

Neuromuscular Coordination of Movement: The work in this project is closely associated with that of "Models of Neural Interactions" (Project Z01 NS 02079-14 LNLC) and they will be described together. These projects include a variety of studies that utilize novel approaches to study motor performance in intact, behaving cats, and to compare actual motor performance with the predictions made by several theoretical model systems that embody general control principles. Much of the experimental data is obtained using novel, chronically-implanted transducer systems that have been developed and perfected in LNLC over the past decade. The overall goal is to obtain information from intact, freely moving animals that can be related to the existing data base that has been accumulated in anesthetized, immobilized, or otherwise reduced preparations, and to refine model systems that test the applicability of particular general control theories. These projects also interface importantly with the two projects already discussed above.

In any work with awake, behaving animals, it is important to stress certain aspects of animal handling that are related to humane concerns. In this work, device implantation is done under anesthesia and aseptic technique, with appropriate postoperative care. Animals are acclimatized to the laboratory situation before surgery and are trained to walk or run on the treadmill, or on a special runway containing force plates, using affection and food rewards. There is no apparent discomfort associated with the implanted devices or the connector system after the initial postoperative period, and the animals display no aversive reactions to the laboratory situation.

A critical factor in understanding the neural control of limb movement is a detailed understanding of the architecture and kinesiology of the individual muscles that are used by the nervous system to regulate

posture and produce movement. We have continued a detailed analysis of the cat hindlimb musculature with particular reference to structure-function interrelations in bi- or multi-articular muscles. The functional role(s) played by such muscles can vary depending on limb position and often do not fit with simple flexor-extensor, or agonist-antagonist, classifications. We have also focussed on the internal architecture of long, very distensible muscles such as the sartorius and tenuissimus, which are made up of serial arrangements of relatively short muscle fibers that interdigitate with one another. The arrangement of muscle fibers within individual muscle units has been studied using the glycogen-depletion method. The in-series muscle fibers within the muscle unit do not appear to be linked in strict serial order but rather transmit longitudinal forces via a complex network of intramuscular connective tissue fascicles. This organization provides a very wide range in physiological length while maintaining the ability of individual sarcomeres to function within their very limited physiological range of movement.

Our understanding of the complexity of coordinated muscle action during limb movement has grown with the increasing sophistication of the computerized data base (cat hindlimb model) being developed jointly by LNL and the Dept. of Electrical Engineering at the University of Maryland (Contract N01-NS-3-2348). This development illustrates very well the utility of a computer model in neuroscience research, not as an end in itself, but rather as a guide for thinking and for perfection of increasingly focussed experiments. To date, simulations of the multiple segment, articulated limb model have shown the critical importance of passive forces (moving masses and gravity) in generating cyclic movement. Limb muscles frequently act to limit movement and recover kinetic energy (i.e., undergo active lengthening), rather than to generate positive work (i.e., active shortening against a load). In some cases, observed muscle action appears anti-intuitive unless considered in the larger context of whole limb kinetics. CNS control of limb movement obviously deals with limb and body kinetics, and it is necessary to consider this context when we attempt to understand the organization of neural control system within the CNS.

Since it is relatively easy to record the electrical activity of muscles (EMG) *in situ* but more difficult to record directly whole muscle force using our implanted strain gauge devices, we have attempted to validate predictions of muscle force output from EMG data. This utilizes a model formulation that involves non-linear terms, including such factors as tendon elasticity, muscle fiber angulation, electromechanical activation delays, and the dependence of force output on muscle fiber length and velocity. Input data are derived from EMG, length, and force records from intact, moving animals, and from measurements of the relevant properties at the sarcomere level in acute, anesthetized cats. Some of the latter work utilizes laser diffraction methods to examine the length excursions of individual sarcomeres in target muscles *in situ* during passive and active states at lengths within the physiological range. The results to date are encouraging in that it appears to be possible to develop a generalized model of the EMG-force relationship for muscle in which internal architecture is accurately known.

We have continued to refine designs for electrodes to record muscle EMG reliably from large and small limb muscles, so as to be able to infer the control strategies being used by the central nervous system during various kinds of natural movements. For example, we have utilized muscles in which there is non-uniform distribution of muscle fiber types (which are associated with different motor unit types; see Project Z01 NS 02160-13 LNL) in order to test prevailing hypotheses about stereotyped versus differential recruitment of the three major motor unit types during a wide variety of natural movements. When the approach is sufficiently validated, it can be used to study recruitment patterns during voluntary (i.e., self-initiated) movements in behaving animals, as well as reflex effects produced in different groups of motor units following stimulation of particular muscle or cutaneous nerves. The approach thus should permit inferences developed by studies in immobilized animals (Project Z01 NS 01686-19 LNL) to be tested in intact, behaving animals.

The problem of neural control of complex muscles is also being investigated in the highly complex muscles that control head and neck movement. This work is done in collaboration with the Department of Physiology, Queens University, Kingston, Canada. Morphological and histochemical studies are done in Canada and the results are compared with physiological and kinesiological observations obtained in LNL,

largely using chronic implant technology. Muscle synergies, reflex effects, and varying degrees of motor unit synchronization are being studied. We have found surprising degrees of motor unit synchronization (at about 25 Hz) during certain types of head movements, while other movements that utilize the same muscle do not show such synchronization. Current experiments suggest that the synchronization is a purely central phenomenon but its functional role remains obscure.

Cortical Mechanisms of Voluntary Motor Control: Work in this project is designed to increase our understanding of the organization of neuronal systems in regions of the cerebral cortex of primates that are associated with the control of voluntary movement and which project directly or indirectly to the spinal cord and brain stem (primarily the sensorimotor cortex and supplementary motor area [SMA]). The major emphasis is on control of arm, wrist, and finger movements. Most of the in-house research is done using non-human primates (rhesus monkeys), although LNL staff members involved in this project are also collaborating with investigators outside NIH on the development of safe and practical methods for intra-cortical microstimulation (ICMS) in human subjects.

In the work with monkeys, animals are intensively trained to perform specific tasks and then chronic recording chambers are implanted over the appropriate areas of the motor or SMA cortex, under anesthesia and strict aseptic technique. During daily recording sessions, which usually extend over many months, the animals are minimally restrained in a primate chair and are rewarded for desired performance with fruit juice. The discharge patterns from individual neurons during voluntary arm and hand movements are recorded in relation to movement performance, as well as during evaluation of the sensory receptive fields by natural stimulation of skin, joint, and muscle receptors. These studies indicate the existence of "colonies" of cortical neurons that produce patterns of excitation and/or inhibition in groups of muscles, rather than in individual muscles. The organization of cortical inhibition, presumably operating through spinal segmental interneuron systems, is of particular interest, since we have found that zones that produce inhibition of target muscles often border, or surround, zones that produce pure excitation. Much of this complexity had been missed in other studies of cortical organization because multi-muscle EMG methods have not been extensively utilized.

Recent work with Prof. Robert Porter, Fogarty Scholar-in-Residence, was undertaken to test the idea that the SMA can influence the response of the primary motor cortex to sensory inputs. A novel cooling probe was used to reduce the activity of SMA in a completely reversible manner. Initial results suggest that cooling the SMA produces no detectable defect in trained movement performance or the response of motor cortical neurons to proprioceptive input.

The new tracer substance, CTX-HRP (see above) has been evaluated in several monkeys for ability to label motoneurons and muscle afferent termination fields. As in the cat and rat, CTX-HRP shows long persistence in monkey tissue (> 1 week) and remarkable labeling of motoneuron dendritic territories. In addition, prolonged survival leads to accumulation of the material in afferent termination sites, not only in the spinal cord but in the brain stem as well. In principle, the material should permit development of detailed information about the regions of spinal cord and brain stem that are associated with individual muscles.

We have continued collaborative work begun in FY 1986 to assess the possible utility of ICMS techniques, already studied extensively in animals, as the basis for motor or sensory prostheses to aid neurologically handicapped patients. LNL staff members have worked in concert with other NIH scientists from BEIB, the Fundamental Neurosciences Program and a team of neurosurgeons in London, Ontario, Canada (University of Western Ontario), to test the effects of ICMS in selected human patients undergoing craniotomy and cortical resection. The aim of this work is to evaluate the quality of sensations perceived by patients during ICMS at stimulus intensities compatible with minimal tissue reaction in animal studies. This work will also utilize improved metal microelectrodes (activated iridium) developed in LNL. The results in the first patient evaluated were extremely encouraging but were less satisfactory in the second two. Further studies are planned on this important issue.

Techniques for Making Contact with the Nervous System: This project includes all LNLC activities related to the development, design, and fabrication of instrumentation, specialized mechanical equipment, and transducer devices used to support the research work of LNLC, as well as the development of computer software necessary to handle multiple simultaneous streams of data from on-line experiments. Virtually all staff members of LNLC participate in one or more aspects of this project, both as an adjunct to their own research and as a way to share the fruits of their efforts with other staff members and projects. Many of the techniques and instruments developed in LNLC are new and without commercial counterpart. In such cases, LNLC staff attempt to provide assistance to other scientists at NIH and at other institutions around the world who request information and advice about specific data acquisition and processing problems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01686-19 LNLC
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Motor Control Systems in the Spinal Cord		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R.E. Burke, M.D.	Chief LNLC, NINCDS
Others:	J.W. Fleshman, Ph.D.	Staff Fellow LNLC, NINCDS
	D.E.R. Meyers, Ph.D.	Visiting Fellow LNLC, NINCDS
	Diane Omeniuk, M.S.	Student Scientist (3)
	Pablo Rudomin, Ph.D.	Fogarty Scholar-in-residence (2)
	Brian J. Schmidt, M.D.	Guest Worker LNLC, NINCDS
	M. Tokuriki, D.V.M.	Guest Worker (1)
COOPERATING UNITS (If any) (1) Dept. of Physiology, Sch. Vet. Med., Yamaguchi Univ., Japan; (2) Dept. of Physiology, CIEA del IPN, Mexico City, Mexico; (3) Dept. of Physiology, George Washington Univ. Sch. Medicine, Washington, DC		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	5.3	PROFESSIONAL: 4.5 OTHER: 0.8
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is designed to provide information on the mechanisms operating within <u>reflex</u> systems in the adult cat spinal cord, which include <u>alpha motoneurons</u> as the output link, as well as on the interconnections and interactions between reflex pathways and control systems descending to the spinal cord from supraspinal centers. Particular consideration is also given to interrelations between <u>synaptic organization</u>, intrinsic neuronal properties, and dynamic behavior of the alpha motoneurons, and the motor unit type, as defined by the <u>physiological</u> characteristics of the innervated <u>muscle fibers</u>. A variety of preparations have been used, including anesthetized or decerebrate animals as well as intact, freely moving cats. Electrophysiological and morphological data are obtained.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01687-19 LNLC
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Techniques for Making Connections with the Nervous and Musculoskeletal Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M.J. Bak Electronics Eng LNLC, NINCDS Others: R.E. Burke, M.D. Chief LNLC, NINCDS; G.E. Loeb, M.D. Med Off (Res) LNLC, NINCDS J.W. Blaszczyk Visiting Fellow LNLC, NINCDS; A.J. Rindos Guest Res LNLC, NINCDS C.M. Chanaud Guest Res. LNLC, NINCDS; E.M. Schmidt, Ph.D. Bio Eng LNLC, NINCDS J.W. DeClaris Student Aid LNLC, NINCDS; S.L. Wallace Student aid LNLC, NINCDS G.M. Dold Eng. Tech LNLC, NINCDS; W.J. Yee Biol Eng LNLC, NINCDS		
COOPERATING UNITS (if any) Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht)		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 0.2	OTHER: 1.4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations. Because of this laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable mechanical transducers, catheters, and connectors. Also included is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01688-19 LNLC
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cortical Mechanisms of Voluntary Motor Control		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: E.M. Schmidt, Ph.D. Others: M.J. Bak G.M. Dold F.T. Hambrecht G.B. Jastrzebski, Ph.D. J.S. McIntosh R. Porter, M.D.	Biological Engineer Electronics Engineer Engineering Technician Guest Researcher Physiologist Fogarty Scholar in Residence	LNLC, NINCDS LNLC, NINCDS LNLC, NINCDS FNP, NINCDS LNLC, NINCDS John Curtin School Med.
COOPERATING UNITS (if any) Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht); Neuroprosthesis Research Program, NINCDS; Univ. Western Ontario, London, Ontario, Canada (Dr. J. Girvin); John Curtin School of Medicine, Sydney, Australia (R. Porter, M.D.)		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 0.8	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is designed to investigate the spatial distribution and functional properties of cortical neuron "colonies" in the primate motor cortex that project to the spinal cord and are associated with individual muscles or closely related groups of muscles, as well as the activity of neurons in such colonies during defined voluntary motor behaviors. <u>Cortical cell discharge patterns</u> during normal movements are evaluated in terms of EMG patterns, and their responses to small loading and unloading torque perturbations. These responses are evaluated before, during and after cooling of the <u>supplementary motor area</u> . Spinal cord location of motoneurons innervating selected forelimb muscles and termination patterns in the spinal cord and brain stem of sensory receptors within these muscles are studied using anterograde and retrograde tracing methods.		
9 - LNLC/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02079-14 LNLC
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Models of Neurophysiological Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: W.B. Marks, Ph.D. Others: Sergio H. Duenas Naotoshi Sugano Research Physiologist LNLC, NINCDS * **		
COOPERATING UNITS (if any) *Centro de Investigaciones Biomedicas, Universidad de Colima, Mexico **Department of Electrical Engineering, Tamagawa University, Tokyo, Japan		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	3.3	0.4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) As quantitative data from a wide variety of techniques and levels of investigation become available for a particular nervous system function, it is both possible and advisable to attempt to assimilate such information into a comprehensive model of the underlying mechanisms and their interactions. This project consists of the development of such models and the necessary <u>analytical and mathematical techniques</u> for their implementation and testing in several areas of intensive experimental investigation by LNLC members and the scientific community at large. Several lines of evidence from walking cats suggest that <u>primary afferents are depolarized</u> at certain moments <u>during stepping</u>, and that this controls the strength of the afferent signals. A <u>model of the motoneuron with after hyperpolarization</u> approximates the response of real motoneurons to varying injected currents. By <u>extending factor analysis</u> we have found the <u>optimum mixture of input signals</u> from color receptors that would be used by ganglion cells of the retina using the criterion that the signals they generate should be independent signals. We have derived a relationship between the firing of synaptic terminals onto a neuron and the <u>rate of information flow into the neuron</u>, when the terminals fire irregularly and the neuron is affected by other terminals at the same time. We have the first evidence that an <u>important feature of the shape of a neuron is its fractal dimension</u>.		
10 - LNLC/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02080-14 LNLCD
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (<i>#0 characters or less. Title must fit on one line between the borders.</i>) Neuromuscular Coordination of Movement *		
PRINCIPAL INVESTIGATOR (<i>List other professional personnel below the Principal Investigator. (Name, title, laboratory, and institute affiliation)</i>)		
PI:	G.E. Loeb, M.D.	Medical Officer, Research LNLCD, NINCLD
Others:	J.B. Blaszczyk, Ph.D.	Visiting Fellow LNLCD, NINCLD
	C.A. Chanaud	Guest Researcher LNLCD, NINCLD
	C.J. Heckman	Guest Researcher LNLCD, NINCLD
	C.A. Pratt, Ph.D.	Staff Fellow LNLCD, NINCLD
	A.J. Rindos	Guest Researcher LNLCD, NINCLD
	J.L.F. Weytjens	Visiting Fellow LNLCD, NINCLD
COOPERATING UNITS (<i>if any</i>) Queen's University, Dept. of Physiology, Kingston, Ontario, Canada (F.J. Richmond); U. of Maryland, Dept. of Electrical Engineering		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCLD, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	5.8	PROFESSIONAL: 3.9 OTHER: 1.9
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (<i>Use standard unreduced type. Do not exceed the space provided.</i>) The cat has long been a standard animal for anatomical and acute physiological studies of muscle function and motor control at the <u>spinal cord</u> level. In this project, a wide variety of traditional and novel <u>kinesiological techniques</u> are being used to study motor tasks in unanesthetized, normally behaving cats, including computer-aided reconstruction of skeletal movement from videotape, multi-axis force plates, chronically implanted nerve cuff and EMG electrodes, and strain and length transducers. The major focus has been the study of <u>hindlimb muscles</u> and their afferent and efferent control during walking, which is the subject of a computer modeling project described in (Project No. Z01-NS-02079-14 LNLCD) and contract narrative No. NS-32348. Other hindlimb movements studied include jumping, paw shaking, scratching, and reflexes to cutaneous nerve stimulation during normal and <u>decerebrate</u> walking. In a collaborative study, similar data are being collected from a large number of <u>neck muscles</u>. The major objective is to correlate patterns of usage with the complex <u>mechanics</u> and compartmentalization and proprioceptive specializations of these muscles. A major theme emerging from these experiments is a concept of "<u>Task Groups</u>," which denotes the segregation and specialization of sensorimotor systems to perform kinematically homogeneous tasks in an optimal manner. This is particularly apparent in <u>multiarticular muscles</u>, which in some cases use independent subdivisions of their alpha motoneuron pool to accomplish kinematically diverse tasks. Recent work has concentrated on the functional architecture of muscle units, including the mechanical arrangements of their constituent fibers, segregation on the basis of histochemical fiber types, and compartmentalized patterns of EMG recruitment during normal motor behaviors. We believe that an overview of these various organizational features of many different motor pools will provide insights into both the functional significance of these features and their implications for the spinal segmental circuitry that regulates and coordinates the various muscles. *(This project is the LNLCD/NINCLD support of contract NS-32340. The project officer is Dr. Gerald Loeb.)		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02160-13 LNLC																					
PERIOD COVERED October 1, 1986 through September 30, 1987																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intrinsic Properties of Motor Units																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R.E. Burke, M.D.</td> <td style="width: 33%;">Chief, LNLC</td> <td style="width: 33%;">LNLC, NINCDS</td> </tr> <tr> <td>Others: J.W. Fleshman, Ph.D.</td> <td>Guest Researcher</td> <td>LNLC, NINCDS</td> </tr> <tr> <td>M. Kalia, M.D.</td> <td>Guest Researcher</td> <td>(2)</td> </tr> <tr> <td>A. Lev-Tov, Ph.D.</td> <td>Visiting Associate</td> <td>(1)</td> </tr> <tr> <td>D.E.R. Meyers, Ph.D.</td> <td>Visiting Fellow</td> <td>LNLC, NINCDS</td> </tr> <tr> <td>C.A. Pratt, Ph.D.</td> <td>Staff Fellow</td> <td>LNLC, NINCDS</td> </tr> <tr> <td>B.J. Schmidt, M.D.</td> <td>Guest Researcher</td> <td>LNLC, NINCDS</td> </tr> </table>			PI: R.E. Burke, M.D.	Chief, LNLC	LNLC, NINCDS	Others: J.W. Fleshman, Ph.D.	Guest Researcher	LNLC, NINCDS	M. Kalia, M.D.	Guest Researcher	(2)	A. Lev-Tov, Ph.D.	Visiting Associate	(1)	D.E.R. Meyers, Ph.D.	Visiting Fellow	LNLC, NINCDS	C.A. Pratt, Ph.D.	Staff Fellow	LNLC, NINCDS	B.J. Schmidt, M.D.	Guest Researcher	LNLC, NINCDS
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COOPERATING UNITS (if any) (1) Dept. of Anatomy, Hadassah Med. School, Jerusalem, Israel (2) Dept. of Pharmacology, Jefferson Med. College, Philadelphia, PA																							
LAB/BRANCH Laboratory of Neural Control																							
SECTION																							
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892																							
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3																					
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☒ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews														
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is designed to provide information on the ranges and distributions of the electrophysiological and morphological characteristics of <u>alpha motoneurons</u> and of the interrelated mechanical, histochemical and morphological properties of the <u>muscle fibers</u> innervated by them (i.e., the muscle unit) in various hindlimb muscles in the cat. Methods used include <u>intracellular</u> recording and stimulation, measurement of mechanical properties of muscles and individual muscle units, <u>neuroanatomical</u> techniques of intracellular staining with horseradish peroxidase, along with conventional and computer-aided methods for reconstruction of extensive neuronal structures from serial histological sections, and computer modeling and data processing. In some experiments, motor unit populations in normal animals are compared with those in animals after various conditioning treatments. Studies of alpha motoneuron properties are included in this project when they are related importantly to the type of muscle unit innervated by the studied cells.																							

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neurobiology
National Institute of Neurological
and Communicative Disorders and Stroke

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October 1, 1986 through September 30, 1987

Laboratory of Neurobiology, DIR
National Institute of Neurological and Communicative
Disorders and Stroke

Thomas S. Reese, M.D., Chief

The Laboratory of Neurobiology has two Sections, the Section on Structural Cell Biology and the Section on Structural Plasticity. The Section on Structural Cell Biology uses modern structural and biochemical techniques to investigate basic cell biological problems germane to an understanding of the function of nerve cells; the Section on Structural Plasticity applies these and other appropriate approaches directly to problems of both fundamental and clinical importance in the mammalian central nervous system, emphasizing problems related to regeneration and response to injury. Current emphasis of the Section on Structural Cell Biology is on the mechanism of axoplasmic transport, axonal growth, and synaptic function while the Section on Structural Plasticity is investigating factors which promote establishment of blood-brain barrier function and neural connections in neural tissues implanted in the brain.

The Section on Structural Cell Biology has recently discovered the molecular basis of the directed organelle movements underlying fast axoplasmic transport. The translocator protein is neither a dynein nor a myosin but, instead, turned out to belong to a new class of motility proteins that we named kinesin. Kinesin (with ATP) is able to mimic the movements serving fast anterograde transport. Kinesin also occurs in many types of non-neuronal cells and appears to be of general significance in cell motility.

Organelle movement induced by kinesin, however, is in only one direction with respect to microtubule polarity. This direction is away from the neuronal cell body, as determined by observing kinesin-induced movement of latex beads along microtubules made from centrosomes (all centrosomal microtubules like those in the neuron have the same defined polarity). Therefore, kinesin must mediate anterograde axonal transport only. Indeed, brain supernatant stripped of its kinesin, with a monoclonal antibody, induces bead movement in a direction that would correspond to retrograde axonal transport. There is, thus, a separate retrograde translocator which has a

higher sedimentation coefficient than kinesin. Since the retrograde translocator does not show the AMP-PMP binding to microtubules characteristic of kinesin, we have not been able to purify it yet.

The movements of microtubules on kinesin-coated glass were analyzed with video microscopy in order to determine how kinesin generates the microtubule-based movements. These movements turned out to depend on the concentration of various nucleotides such as ATP, metal ions, and of kinesin itself. This approach enables precise manipulation of the chemical motor powering movement using movement itself as the endpoint. The results to date indicate that kinesin-based motility has many properties consistent with it being an ATPase, while other aspects of the mechanobiology differ from the known properties of myosin and dynein, the other two proteins which mediate intracellular motility. We have also developed a method for visualizing in the electron microscope regions of kinesin-coated glass where microtubule movements were previously observed in the video microscope. By combining these biophysical and structural approaches, we hope to establish how kinesin transduces its chemical energy into movement. Our most recent kinetic data suggests that kinesin has two interacting binding sites for ATP.

Another new approach has been to analyze the kinesin induced movements of beads on microtubules with computer-based analyses that permit movements as small as 20nm to be defined. The results suggest that beads follow the protofilaments in microtubules, because the moving beads show a lateral shift that corresponds to the pitch of the microtubule protofilaments. Bead movement is also discontinuous, showing a quantal characteristic with subunits that might correspond to individual kinesin-induced events. This new technique of motion analysis is expected to yield a variety of new insights on how kinesin induces movements, and to be applicable to the myosin and dynein-based systems as well.

The work on kinesin has also extended to a consideration of other organelle proteins that might be involved in kinesin-based motility. Organelles isolated from axons are no longer moved by kinesin when they are stripped of extrinsic proteins by high salt treatment; however, certain fractions of the axonal supernatant proteins will restore the ability of kinesin to move organelles along microtubules. We are currently attempting to isolate and characterize the ancillary proteins involved in movement of organelles in the axon.

Direct freezing and improved freeze-substitution techniques have also been applied to growing tips of neuronal processes during development of synaptic connections in the chick optic tectum. Numerous flattened vesicles are found in groups near the growth cone surface; their total area exceeds that of the plasmalemma. A large pool of internal membrane is therefore potentially available to support the rapid expansion of the growth cone surface. We have now shown that these membranes can join the surface membrane at the tip of the growth cone, and that material internalized from the surface ends up in the vicinity of these vesicles. A system for recycling membrane through the plasmalemma of the elongating axonal tip is defined by these studies; furthermore, we have developed a detailed picture of the pathways for recycling of membrane through the growth cone.

The serial reconstruction of growth cones also provided reconstructions of individual microtubules. Organelles contacting microtubules were, judging from the results with the squid axon, in transport. The distribution of organelles was uniform along microtubules in axons and proximal growth cones. However, in the mid-segment of the growth cone the frequency of the small and then the large organelles abruptly dropped, even though the microtubules extended to the ends of the growth cone. Thus, there appear to be discrete loading and unloading zones for axonal transport along the shafts of microtubules in the growth cone.

Growth of the axon also depends on the arrival of cytoskeletal and cytoplasmic elements made in the cell body; this process of slow axonal transport continues even in adult axons. Our previous work on a plant cell, Nitella, led to developing a model of how the axon might move its cytoplasm. This model relies on the smooth endoplasmic reticulum to couple the cytoplasm to the axonal membrane. To study this model we have developed a method to isolate endoplasmic reticulum from squid axoplasm and have shown that it is coated with myosin. The next step is to see whether this myosin can produce movement of the endoplasmic reticulum when it is brought into contact with actin filaments, such as those lining the inner surface of the axon membrane.

A set of projects depending on more specialized cryotechniques -- direct freezing, cryosectioning, electron microscopic immunocytochemistry, quantitative x-ray microanalysis, and elemental x-ray imaging -- have yielded new information on the distribution of diffusible elements, including calcium, in cerebellar synapses, and of structural and transport proteins in axons and glia. The x-ray imaging had previously shown that high-calcium sequestration sites were present in highly stimulated preparations only in some of the Purkinje cell dendritic spines. A thorough, quantitative elemental analysis of stimulated and resting cerebellar synapses has now characterized Ca-sequestering organelles operating at normal, regulatory calcium levels. Parallel fiber synaptic vesicles contain less than 0.7 mmol/kg wet weight total calcium in both resting and stimulated preparations, but elevated internal calcium (five-to-six fold in stimulated synapses and reflecting typical sequestration activity) was found in presynaptic smooth endoplasmic reticulum and in the smooth-membrane cisterns of dendritic spines. These results indicated a role for neuronal endoplasmic reticulum as a calcium-regulatory organelle and, further, imply the involvement of calcium in the formation and maintenance of specialized synaptic contacts.

The development of cryosectioning techniques for x-ray microanalysis led to its application in electron microscopical immunocytochemical methods for localizing cytoskeletal proteins in frozen sections of nervous tissues; this new approach was initially applied to a study of myelin structure and development and then to interactions of axons and glia. The results have implicated several structure and transport proteins, e.g., actin, spectrin and kinesin, in the synthesis, intracellular transport, and insertion of the myelin-specific proteins P_0 and myelin-associated glycoprotein (MAG). Further, a new cytochemical approach, applying in situ hybridization methods to cryosections, has been developed and used to demonstrate differences in the distributions of the m-RNAs encoding for the central nervous system myelin proteins, the myelin basic protein and the proteolipid protein.

Section Summary

The Section on Structural Plasticity has been involved in three projects: (a) to test the notion that we have raised as to whether neuroendocrine axons, whose normal targets are blood vessels, express neurovascular specificity; (b) to determine whether the shedding of apical cytoplasm from choroid plexus epithelium takes place within the intact organism; and (c) to further characterize the particle assemblies inside the cell membrane of astrocytes, and to define the mechanisms of signal transduction involved in the proliferation of these cells.

As with a muscle or neural target, do regenerating axons end specifically upon or very near the wall of blood vessels with which they normally, closely associate? Neurosecretory axons (NSA) end near fenestrated blood vessels (FBV) in the pituitary's neural lobe. Such vessels are passively permeable to the peptide hormones released by the NSA. Do mature, regenerating NSA end selectively on the FBV of the pituitary's neural lobe, or can they associate as closely with the FBV of other organs? We have taken advantage of our earlier finding that grafted blood vessels from peripheral tissues to the brain not only survive, but retain their morphological and permeability characteristics. Fragments of neural lobe, pineal gland and adrenal medulla were transplanted bilaterally to the retrochiasmatic area of the rat hypothalamus where the NSA fasciculate and where there are no FBV. Although the anterior part of the rat's pineal gland receives a few vasopressin fibers, the adrenal medulla does not. Two and 4 weeks after the transplantation, a few regenerating NSA enter the adrenal and pineal grafts and some of these came to lie as closely (0.5 - 4.0um) to FBV as they did in the normal and grafted neural lobe. There does not appear to be specificity of association between these neuroendocrine axons and fenestrated capillaries. However, many more NSA grew into the neural lobe grafts than did into the other two types of tissue. The pituitary and its extracellular products in the neural lobe are the most likely elements that are tropic for the NSA. Experiments are contemplated to see whether these cells not only attract and induce regeneration of NSA, but axons from other sources as well.

Isolated choroid plexus (CP) epithelium has been recently found to shed, in vitro, its apical cytoplasm. Membrane bounded spheroids, or "aposomes", about 2-9um wide, and containing, primarily, free ribosomes are extruded. Aposomes can incorporate labeled amino acids. We have asked whether aposomes are released by CP in vivo, where they may be carried by the cerebrospinal fluid (CSF) circulation, and what are the proteins that they synthesize? The CSF of adult rats was examined for aposomes by centrifuging small samples onto glass slides. Aposomes were detected immunohistochemically and by their size, their shape having been distorted by the deposition method. An antibody to transthyretin, which is produced by CP epithelium, and a donated, monoclonal antibody (C3.5) raised against a crude fraction of aposomes, stained particles in the CSF samples. The size of the particles were in the range of aposomes. A few, transthyretin and C3.5 - positive particles reached the deep cervical lymph nodes as detected in cryosections of unfixed, frozen tissue. If we find that aposomes bind antibodies to Ia antigen, they could qualify as antigen presenting units. In order to identify the proteins made by the aposomes, we harvested aposomes from isolated CP taken from the same rats whose CSF had been sampled. The aposomes were maintained for an hour in simulated CSF to which ³⁵S-labeled methionine and cysteine had been added. Trichloroacetic acid

precipitable counts were made over the labeling period. The remaining sample was processed by 2-dimensional SDS - gel electrophoresis in order to separate their synthesized proteins, which were visualized by silver staining followed by radioautography. Alternatively, the proteins were identified with the antibodies according to standard immunoblotting. The aposomes produced considerably less transthyretin than did whole CP. The two major proteins synthesized by the aposomal fraction have not, as yet, been identified. We have not yet determined whether a protein, produced by whole CP, which has a molecular weight of 180K daltons and a pI of 6.5, is also synthesized by aposomes. Thus aposomes can be elaborated by CP in vivo, can reach the deep cervical lymph nodes and might, consequently, be involved in immunological responses, and can synthesize proteins which are a subset of those made by CP. The aposomal subset is deficient in transthyretin production but still has vigorous protein synthesis.

We have demonstrated how the astrocyte, as the closest neighbor to cerebral endothelium, can modify the structure of the endothelial cell membrane. We are now determining whether the astrocyte's plasma membrane can, in turn, be modified by endothelial cells. Secondary cell cultures, enriched in astrocytes, as monitored by immunostaining with antibody to glial fibrillary acidic protein, were derived from 2 day old rats. The cells were maintained alone or with other cell types, including bovine brain endothelial cells, rat meningeal cells, rat fibroblasts, rat cerebellar granule cells, bovine pulmonary and aortic endothelial cells, and bovine trachea-derived cells. The cultures were fixed, freeze-fractured and the number of assemblies per unit area of membrane counted. The assembly number was consistently and specifically increased 3-6 fold when brain endothelial cells, meningeal cells, or fibroblasts were present. The effectiveness of fibroblasts is not surprising because (a) they are part of the adventitia of muscle-coated blood vessels and thus close to perivascular astrocytes in vivo; and (b) meningeal cells are a specialized type of fibroblast, and the assembly number is very high at the pial-glial interface. The other 3 cell lines had no effect. As astrocyte processes satellite to neuronal processes in vivo have relatively few assemblies, it was expected that co-cultures with neurons might lead to an actual decrement in assembly concentration. However, the intrinsic, cerebellar astrocytes were carried along with the granule cells to the culture dish, so that assessment must wait for seeding with peripheral neurons or central, cell-sorted ones. A second kind of manipulation was with agents that affect cell metabolism. Forskolin and isoproterenol both augment cyclic adenosine monophosphate (cAMP) levels in astrocytes. Incubation of solo astrocyte cultures with a single pulse of forskolin in 2 experiments raised the assembly number 15 fold after 3 days of incubation. The beta-adrenergic agonist, isoproterenol, which also raises the level of cAMP, had a smaller effect which was augmented when 3-isobutyl-1-methyl-xanthine, which inhibits cAMP degradation, was included. These results suggest that cAMP may play a role in determining the number of assemblies in different physiological states. The mitogen-stimulated proliferation of C6 glioma cells and astrocytes from a human cell line stimulation, was accompanied by the formation of typical isomers of phosphoinositol (PI) degradation. These products included glycerol phosphate inositol diphosphate and glycerol phosphate inositol phosphate, which are rarely found in non-glial cells. In C6 cells, alpha-adrenergic and serotonergic stimulation increased the amount of the PI intermediates. Thus, astroglial proliferation is accompanied by stimulation of the PI cycle. One of the possibilities to be investigated is whether astrocyte proliferation is also mediated by mechanisms other than the PI cycle, e.g., through phosphatylcholine degradation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01442-21 LN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Permeability of Cellular Layers in the Vertebrate Nervous System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Others:	T.S. Reese, M.D., S.B. Andrews, Ph.D. J. Frokjaer-Jensen, Ph.D. B. Kachar, M.D.	Chief Special Expert Visiting Associate Visiting Associate LN, NINCDS LN, NINCDS LN, NINCDS LN, NINCDS
COOPERATING UNITS (if any) ~~Marine Biological Laboratory, Woods Hole, MA 02543~~ J.S. Handler, KE, IR, NHLBI, NIH, Bethesda, MD R.P. Rand, Brock University, St. Catherine's, Ontario, Canada R.C. Wagner, University of Delaware, Newark, DE		
LAB/BRANCH Laboratory of Neurobiology		
SECTION Section on Structural Cell Biology		
INSTITUTE AND LOCATION NINCDS, NIH, The Marine Biological Laboratory, Woods Hole, MA 02543		
TOTAL MAN-YEARS 0.5	PROFESSIONAL 0.5	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The substructure of tight and gap junctions is investigated by direct freezing techniques that avoid any chemical fixation and serve to increase the definition of individual membrane components. A new model of tight junction structure was developed which replaced the previous view these junctions are comprised of rows of intramembrane proteins; rod-shaped structures seen after direct freezing are now interpreted as inverted cylindrical micelles of membrane lipids. Evidence for this model has been gathered from investigations of pure lipid bilayer systems which are induced to form non-planar micellar phases by addition of calcium ion. Cylindrical lipid micelles identical to the cylinders at tight junctions are found embedded in these lipid bilayers. Tight junctions, but not septate junctions, in invertebrates also appear to have lipidic backbones. How tight junctions prevent small charged solutes from entering the brain (across the blood-brain barrier) is made clear by this new model of tight junction structure. Gap junctions form within minutes of treating cells with certain mild lipid solvents, suggesting that precursors of the intramembranous components of gap junctions are continuously present in the cell membrane. Direct freezing techniques were also used to investigate the distribution of endothelial vesicles and other intracellular organelles in capillaries that had not been subjected to chemical treatment or fixation. Approximately threefold fewer apparently free vesicles were found in directly frozen endothelial cells, as compared with aldehyde-fixed cells, from capillaries of the eel swim bladder and also from cultured human endothelium. However, ultrathin serial sectioning showed that in both cryofixed and chemically fixed eel capillaries, as in the capillaries of the frog mesentery, virtually all vesicles are in continuity with the plasma membrane. 6-LN/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01881-17 LN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Structural Basis of Synaptic Transmission And Development *		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P.I.:	T.S. Reese, M.D.	Chief LN, NINCDS
Others:	T. Cheng, Ph.D.	Visiting Fellow LN, NINCDS
	B. Kachar, M.D.	Visting Associate LN, NINCDS
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA 02543 D. Landis, Dept. of Neurology, Massachusetts General Hospital, Boston, MA		
LAB/BRANCH Laboratory of Neurobiology		
SECTION Section on Structural Cell Biology		
INSTITUTE AND LOCATION NINCDS, NIH, The Marine Biological Laboratory, Woods Hole, MA 02543		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.9	1.9	1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Growing nerve terminals in the brain have been reconstructed from serial sections of freeze-substituted brain. These new preparative methods have revealed an internal system of membranes which are thought to be the source of the new membrane added to the surface of the growth cone during its growth. These membranes are highly labile and are destroyed by conventional fixatives. Our new evidence, based on uptake of structural tracers, shows that these membranes participate in recycling of the membranes needed for extension of the growth cone and also function as intracellular compartments where the plasmalemmal surface of the growth cone can be modified during neuronal development. Other evidence from video analysis of axonal outgrowth of cultured neurons indicates that axonal outgrowth is derived from the proximal growth cone and the growth cone plasmalemma is, in part, derived from the anterogradely transported vesicles and vacuoles which move next to and then fuse with it. We have also performed shallow-etch structural analysis of adult synapses in cerebellar cortex and discovered that a meshwork of filamentous proteins surrounds synaptic vesicles near the axon. Molecular shadowing of pure synapsin I tentatively identifies this protein as a major component of the presynaptic meshwork. * (Formerly, "Structural Basis of Synaptic Transmission")		

7-LN/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02551-06 LN																		
PERIOD COVERED <u>October 1, 1986 through September 30, 1987</u>																				
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) <u>Structure of Neuronal Cytoplasm</u>																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; margin-top: 10px;"> <tr> <td style="width: 33%;">P.I.: T.S. Reese, M.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LN, NINCDS</td> </tr> <tr> <td>Others: T.P.O. Cheng, Ph.D.</td> <td>Visiting Associate</td> <td>LN, NINCDS</td> </tr> <tr> <td>P. Gallant, Ph.D.</td> <td>Special Expert</td> <td>LN, NINCDS</td> </tr> <tr> <td>B.J. Schnapp, Ph.D.</td> <td>Staff Fellow</td> <td>LN, NINCDS</td> </tr> <tr> <td>M. Terasaki, Ph.D.</td> <td>Staff Fellow</td> <td>LN, NINCDS</td> </tr> <tr> <td>B. Kachar, M.D.</td> <td>Staff Fellow</td> <td>LNO, NINCDS</td> </tr> </table>			P.I.: T.S. Reese, M.D.	Chief	LN, NINCDS	Others: T.P.O. Cheng, Ph.D.	Visiting Associate	LN, NINCDS	P. Gallant, Ph.D.	Special Expert	LN, NINCDS	B.J. Schnapp, Ph.D.	Staff Fellow	LN, NINCDS	M. Terasaki, Ph.D.	Staff Fellow	LN, NINCDS	B. Kachar, M.D.	Staff Fellow	LNO, NINCDS
P.I.: T.S. Reese, M.D.	Chief	LN, NINCDS																		
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M. Terasaki, Ph.D.	Staff Fellow	LN, NINCDS																		
B. Kachar, M.D.	Staff Fellow	LNO, NINCDS																		
COOPERATING UNITS (if any) M. Sheetz, Washington University, St Louis, Mo. T. Schroer, Washington University, St. Louis, Mo. R. Vale, Ph.D. University of California, San Francisco, CA.																				
LAB/BRANCH Laboratory of Neurobiology																				
SECTION Section on Structural Cell Biology																				
INSTITUTE AND LOCATION NINCDS, NIH; The Marine Biological Laboratory, Woods Hole, MA 02543																				
TOTAL MAN-YEARS 5.0	PROFESSIONAL: 3.0	OTHER: 2.0																		
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project determines the structure of neuronal and glial cytoplasm, particularly as it pertains to axoplasmic transport, and the organization of cytoplasm. Cultured myocytes grown on grids, frozen, freeze-substituted, and examined directly at high voltages in an electronmicroscope have a cytoplasmic ground substance consisting of fine filaments instead of a microtubular meshwork, and distinct cytoplasmic domains characterized by organelle movements along microtubules. Microtubules isolated from the axoplasm of the squid giant axon continue to support movements of organelles for many hours. However, actin filaments in other cells support similar movements of organelles. A protein translocator responsible for these organelle movements has been characterized, a 600 KD protein with 110 KD and 60-65 KD doublet peptides. This protein induces beads to move along purified microtubules in the presence of ATP. When a monoclonal antibody column (directed towards the 110 KD subunit) is used to purify this translocator it has the same peptide components and supports similar movements. Based on its size, pharmacological properties, and asymmetrical shape seen by molecular shadowing, this translocator protein belongs to a new class of motility protein, which we call <u>kinesin</u>. Kinesin appears to be of general significance in cellular motility. The organelle movements induced by kinesin are always directed towards the plus ends of microtubules, a direction corresponding to <u>anterograde</u> axonal transport. However, brain extracts stripped of kinesin with <u>monoclonal</u> antibody induce bead movement in the <u>retrograde</u> direction. Our current efforts are concentrated on purifying the translocator for retrograde movements and on determining whether organelles selectively bind translocators in order to explain how they are selected for anterograde or retrograde transport. 8-LN/DIR																				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02610-04 LN									
PERIOD COVERED <u>October 1, 1986 through September 30, 1987</u>											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) The Distribution of Mobile and Structural Components at Chemical Synapses											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.</td> <td style="width: 33%;">S.B. Andrews, Ph.D.</td> <td style="width: 33%;">Special Expert LN, NINCDS</td> </tr> <tr> <td>Others:</td> <td>T.S. Reese, M.D.</td> <td>Chief LN, NINCDS</td> </tr> <tr> <td></td> <td>K.G. Herman, Ph.D.</td> <td>Staff Fellow LN, NINCDS</td> </tr> </table>			P.I.	S.B. Andrews, Ph.D.	Special Expert LN, NINCDS	Others:	T.S. Reese, M.D.	Chief LN, NINCDS		K.G. Herman, Ph.D.	Staff Fellow LN, NINCDS
P.I.	S.B. Andrews, Ph.D.	Special Expert LN, NINCDS									
Others:	T.S. Reese, M.D.	Chief LN, NINCDS									
	K.G. Herman, Ph.D.	Staff Fellow LN, NINCDS									
COOPERATING UNITS (if any) C.E. Fiori and R.D. Leapman, BEIB, DRS, NIH, Bethesda, MD. D.M.D. Landis, Case-Western Reserve University, Cleveland, OH. B.D. Trapp, Johns Hopkins University School of Medicine, Baltimore, MD.											
LAB/BRANCH Laboratory of Neurobiology											
SECTION Section on Structural Cell Biology											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892 The Marine Biological Laboratory, Woods Hole, MA 02543											
TOTAL MAN-YEARS	PROFESSIONAL	OTHER									
2.4	1.4	1.0									
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) This project aims to determine the intracellular distribution of diffusible and structural components within axons, dendrites, glia, and synapses. This work is important because of the relationship between the localization and movement of cellular constituents and their role in synaptic transmission. Quantitative x-ray microanalysis of the intracellular distribution of calcium and other elements in parallel fiber/Purkinje cell cerebellar synapses indicates that among all the pre- and postsynaptic organelles only the smooth endoplasmic reticulum accumulates significant calcium under physiological conditions, and that this accumulation occurs from extracellular sources as a function of synaptic activity. The amount of calcium taken up in the cisterns of the Purkinje cell dendritic spines -- a threefold increase from 700 ions to 2100 ions -- is consistent with calcium entry into spines via a ligand-gated ion channel. Complementary new projects using visual system models of neurons and glia have begun to yield data on the identity and activity of intracellular calcium stores. Immunocytochemical studies using frozen sections of actively myelinating peripheral nerve at the electron microscope level have demonstrated the co-localization of the cytoskeletal proteins actin and spectrin and the Schwann cell adhesion molecule MAG in specific cytoplasmic domains of the Schwann cell. These results support a mechanism for myelin sheath formation involving MAG-mediated myelin attachment directed by cytoskeletal motility. Thus, this project continues to provide new information on the detailed relationship between the diffusible and structural components of neurons and glia, and how these regulate neuronal activity.											
9-LN/DIR											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02700-02 LN								
PERIOD COVERED October 1, 1986 through September 30, 1987										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Mechanochemistry of Proteins Involved in Axonal Transport										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">P.I.</td> <td style="width: 40%; vertical-align: top;">B.J. Schnapp, Ph.D.</td> <td style="width: 25%; vertical-align: top;">Senior Staff Fellow</td> <td style="width: 20%; vertical-align: top;">LN, NINCDS</td> </tr> <tr> <td style="vertical-align: top;">Others:</td> <td style="vertical-align: top;">S.H. Kahn, Ph.D. T.S. Reese, M.D.</td> <td style="vertical-align: top;">Guest Researcher Chief</td> <td style="vertical-align: top;">LN, NINCDS</td> </tr> </table>			P.I.	B.J. Schnapp, Ph.D.	Senior Staff Fellow	LN, NINCDS	Others:	S.H. Kahn, Ph.D. T.S. Reese, M.D.	Guest Researcher Chief	LN, NINCDS
P.I.	B.J. Schnapp, Ph.D.	Senior Staff Fellow	LN, NINCDS							
Others:	S.H. Kahn, Ph.D. T.S. Reese, M.D.	Guest Researcher Chief	LN, NINCDS							
COOPERATING UNITS (if any) <table style="width: 100%; border: none;"> <tr> <td style="width: 20%;"></td> <td>M.P. Sheetz, Washington University, St. Louis, Mo.</td> </tr> <tr> <td></td> <td>J. Gelles, Washington University, St. Louis, Mo.</td> </tr> <tr> <td></td> <td>Albert Einstein College of Medicine, Bronx, NY</td> </tr> </table>				M.P. Sheetz, Washington University, St. Louis, Mo.		J. Gelles, Washington University, St. Louis, Mo.		Albert Einstein College of Medicine, Bronx, NY		
	M.P. Sheetz, Washington University, St. Louis, Mo.									
	J. Gelles, Washington University, St. Louis, Mo.									
	Albert Einstein College of Medicine, Bronx, NY									
LAB/BRANCH Laboratory of Neurobiology										
SECTION Section on Structural Cell Biology										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892										
TOTAL MAN-YEARS	2.2	PROFESSIONAL 1.2 OTHER 1.0								
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this new project is to understand how the motors which power fast axonal transport transduce the chemical energy associated with the hydrolysis of ATP into directed movement of organelles along microtubules. We are analyzing the sliding of microtubules along glass coated with kinesin, the protein which powers anterograde transport and analyzing the motion of plastic beads coated with either kinesin or the retrograde translocator in order to determine the sequence of chemical reactions which comprise the mechanochemical cycle; the different structural configurations which kinesin and the retrograde translocator undergo during the work cycle; and how the chemical cycle is coupled to the work cycle. The heart of this project involves analyzing microtubule based motility by video microscopy, using a digital processor to generate images with sufficient contrast to visualize single microtubules and to acquire and analyze motion as a function of manipulations of the chemical environment. This information complements biochemical measurements including binding of microtubules and nucleotides to the translocators and ATPase activities. Electron microscopy of rapidly frozen kinesin on glass or plastic beads is used to determine structural configurations of transient intermediates. These investigations define fundamental biophysical properties of kinesin and retrograde translocator-based movement which will serve as a baseline for assaying the effects of other proteins in cytoplasm which interact with and perhaps regulate the motors. Finally, our ability to combine, in a single experiment, biochemistry with motion analysis and electron microscopy using purified components promises to make a major contribution toward understanding the fundamentals of force transduction in motility systems. 10-LN/DIR										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 NS 01805-19 LN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Astrocyte-Endothelial Interactions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P.I.: J.H. Cheng, Ph.D. J.P. Bressler, Ph.D. M.W. Brightman, Ph.D.	Senior Staff Fellow Senior Staff Fellow Head, Section on Structural Plasticity	LN, NINCDS LN, NINCDS LN, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurobiology		
SECTION Brain Structural Plasticity		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 2.4	PROFESSIONAL 1.6	OTHER 0.8
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)		
Astrocytes have characteristic intramembranous particle assemblies. Their distribution is polarized <u>in vivo</u>, with the highest concentration in the astroglial membranes facing blood vessels, pia and the lowest satellite to neurons. To test whether the apposing blood vessels, pia and neurons affect the assembly number, astrocytes from neonate rats were cultured alone or with different cell types and examined with freeze-fracture electron microscopy. Co-culturing astrocytes with brain endothelium, meningeal cells or fibroblasts consistently increased the assembly number 3-6 fold over a 6-17 day period. Bovine pulmonary artery, aortic endothelium and a tracheal-derived cell line were ineffective. The effect of CNS neurons could not be assessed because they could not be separated from their intrinsic astrocytes. Agents that elevate the activity of cyclic adenosine phosphate (cAMP), augmented the assembly number most dramatically. A single pulse of forskolin, which raises cAMP levels in astroglia, increased the assembly number 15 fold after 3 days. The beta agonist, isoproterenol, which also raises cAMP levels in astrocytes, also increased assemblies, especially when 3-isobutyl-1-methyl-xanthine, which inhibits cAMP degradation, was included. These results suggest that cAMP may be linked to the presence of assemblies in the astroglial cell membrane. During mitogen stimulation of C6 glioma and a human cell line of glial cells <u>in vitro</u>, the typical isomers of phosphatidylinositol (PI) degradation were formed. These products included glycerol phosphate inositol diphosphate and glycerol phosphate inositol phosphate, which are rarely found in non-glial cells. In C6 cells, alpha-adrenergic and serotonergic stimulation increased the amount of the PI intermediates. Astroglial proliferation is accompanied by stimulation of the PI cycle. 11-LN/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02086-14 LN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Regeneration Specificity in Transplanted Neural Tissue*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) P.I.: Y. Kadota, M.D. Others: M. Brightman, PhD. K. Pettigrew, PhD. Visiting Fellow LNC, NINCDS Head, LN, NINCDS Section on Structural Plasticity Guest Worker, NIMH, NINCDS Research Mathematical Statistician		
COOPERATING UNITS (if any) N.I.M.H., Theoretical Statistics and Mathematics Branch Laboratory of Neurochemistry, NINCDS		
LAB/BRANCH Laboratory of Neurobiology		
SECTION Section on Structural Plasticity		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 2.8	PROFESSIONAL 1.8	OTHER 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Does a regenerating, neuroendocrine axon, which normally terminates near blood vessels, express neurovascular specificity? The intact neurosecretory axons (NSA) of the basal hypothalamus end near fenestrated blood vessels (FBV) of the pituitary gland's neural lobe. FBV are permeable to the peptide hormones released by the NSA. Will mature, regenerating NSA associate more closely with the FBV of grafted neural lobe than the FBV of other endocrine tissues, such as the pineal gland and adrenal medulla? Pieces, about 0.5mm³, of these 3 tissues were placed bilaterally in the retrochiasmatic area where the NSA are bundled and where there are no FBV. Two and 4 weeks later, the brains were processed for immunohistochemical localization of NSA with a monoclonal antibody to vasopressin neurophysin and for electron microscopy. A few regenerating NSA entered grafted pineal and adrenal medulla. Some of these NSA came to lie within about 0.5-2.0 um of the capillary wall. The NSA - FBV distance is within the range found in normal and grafted neural lobe. This distance was the criterion for NSA - FBV association because there is no synaptoid or other membrane specialization to mark the association. Considerably more (0.05 > p) NSA entered the neural lobe grafts at both 2 and 4 weeks than grew into the pineal and adrenal medulla tissue. We conclude that (a) mature, regenerating NSA are capable of forming close associations with FBV other than their normal targets, and (b) neural lobe tissue, most likely its pituicyte component, has a markedly tropic effect on these axons. * (Formerly: "Regeneration in Transplanted Peripheral and Central Neurons")		

12-LN/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02144-13 LN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Blood-Brain Barrier		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) P.I.: D.Gudeman, B.S. Other: M.Brightman, PH.D. Guest Worker CNB, NIMH Head, LN, NINCDS Section on Structural Plasticity		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurobiology		
SECTION Section on Structural Plasticity		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 3.0	PROFESSIONAL 1.5	OTHER 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Does the extrusion of plasma membrane-enclosed vesicles from the apical surface of isolated choroid plexus (CP) also take place in the intact rat? Two approaches were used. First, the morphological detection of the apically derived vesicles (aposomes) was made in cerebrospinal fluid (CSF) and deep cervical lymph nodes. These lymph nodes were examined because they are part of a major pathway of CSF drainage and it was thought that the nodes would filter out the aposomes. The aposomes were identified immunohistochemically with commercially obtained antiserum to human transthyretin which, in the brain, is synthesized exclusively by the CP and by a donated monoclonal antibody (C3.5) raised against a crude preparation of aposomes and shown to be specific for CP and ependyma. The presence of aposomes in the CSF and deep cervical lymph nodes of the intact rat lend credence to the hypothesis that aposomes are elaborated and released <u>in vivo</u> and suggest that they might be involved in immune responses. Second, protein synthesis in isolated aposomes was described by 2-dimensional SDS-PAGE. Labeled amino acids were used to mark the proteins that were being synthesized and immunoblotting procedures were used to identify important proteins. It was thus determined that, unlike entire choroid plexus tissue, isolated aposomes do not synthesize large amounts of transthyretin but do synthesize two other unidentified proteins. This partitioning of protein synthesis implies a directed cellular sorting by the aposomes and that the selected protein synthesis may have an important bearing on the, as yet, unknown function of aposomes.		

13-LN/DIR

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neurochemistry
National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neurochemistry, Division of Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke
Harold Gainer, Ph.D., Chief

The laboratory is concerned with the development and functional organization of the nervous system, with a specific focus on molecular mechanisms. In addition, the laboratory studies the interactions of the nervous system with various non-neuronal systems (e.g., the endocrine system). The approach of the laboratory is cell biological in nature and, hence, utilizes a wide variety of techniques and concepts from a number of disciplines, e.g., physiology, biochemistry, morphology, immunology, and molecular biology. Specifically, we study neuropeptides and other neurotransmitters, plasma membrane and intracellular membrane systems, cytoskeletal proteins, and various enzymes (e.g., Na,K-ATPase, protein kinases, and proteases) which are found in the nervous system and are essential to its function. A special emphasis is placed on the study of the cellular development of these organ systems.

The Laboratory of Neurochemistry is composed of two Sections: Cellular and Developmental Neurobiology, and Enzyme Chemistry.

I. Section on Cellular and Developmental Neurobiology

Studies on the role of neuropeptides in nervous system development and function, and the molecular mechanisms underlying the unique morphologies of neurons constitute the major activities in this Section. A large number of neuropeptides have been identified as chemical transmitters of information in the nervous system. These known peptides (and presumably those still to be discovered) act as conventional neurotransmitters in a synaptic, paracrine, or autocrine fashion, as well as in neuroendocrine systems. In addition to the well-known hypophysiotrophic regulatory peptide hormones (e.g., corticotrophin releasing factor, luteinizing releasing hormone, thyrotrophin releasing hormone, etc.), there are neuropeptides involved in a wide variety of other CNS functions (e.g., pain, blood pressure control, memory, etc.). This Section's goal is to study the cell biology of peptidergic neurons in the context of their regulatory functions in the nervous system. In particular, we specifically study the expression of neuropeptides during CNS development and their impact on the development of organismic functions and morphology. The second objective has been to study the role of specific macromolecules

in the organization of neuronal structures (e.g., axons, dendrites, spines, and nerve terminals). In this area, we have focused on the neurofilament proteins because of their involvement in axonal structure and Alzheimer's disease, as well as a number of enzymes and proteins involved in calcium and phosphate metabolism.

Our neuropeptide studies have focused on four specific peptidergic neuronal systems in the rodent hypothalamus, the oxytocin, vasopressin, LHRH and CRF neurons. We chose to concentrate on these systems because the neurons that constitute them represent excellent models of peptidergic neurons in the nervous system. The hypothalamo-neurohypophyseal system is studied because the neurons that constitute it (i.e., the oxytocin and vasopressin magnocellular neurons) are prototypic of peptidergic neurons in the central nervous system. These neurons populate two defined topographic sites in the brain (the paraventricular and supraoptic nuclei), and have a specific axonal pathway and termination site, all of which are accessible to experimental manipulation in vivo by stereotaxic, morphological, and biochemical-pharmacological techniques. Access to all three of the critical neuronal structures, i.e., the cell body, the axon, and the terminal in the hypothalamus, median eminence, posterior pituitary, respectively, permits a cell biological analysis of this system. The cell bodies in the hypothalamus are responsible for the biosynthesis of the vasopressin (AVP) and oxytocin (OT) prohormones and peptides. In addition to examining these neurons in the mammal (rat and mouse), we have extended these studies to the neurons that synthesize the evolutionarily related vasotocin (AVT) and mesotocin (MT) peptides in the frog (*Xenopus laevis*). A second peptidergic system under study in the laboratory is the LHRH system in the rat. The LHRH neurons found in the hypothalamus project principally to the median eminence. The secretion of LHRH into the portal blood system regulates the development and function of reproductive system. In addition to being the objects of cell biological studies, the embryonic development of these two neuronal systems is currently also under intensive study. The corticotropin releasing factor (CRF) system projects to the external zone of the median eminence, where the secretion of CRF into the portal system causes the release of ACTH from corticotropes in the anterior pituitary. We are particularly focusing on the co-existence of AVP in the CRF neurons, and the modulation of AVP biosynthesis and release during adrenalectomy and stress.

Progress in the program on peptidergic neuron development and function is as follows: Dr. M. Altstein has studied the development of the AVP and OT neuronal system by quantitative radioimmunoassay methods, and has shown that AVP precedes OT expression by 6 days in embryonic development, and that OT expression is a postnatal phenomenon. In addition, she has evaluated the cause of this differential development and found that both AVP and OT prohormones are expressed concurrently but that the OT

processing is delayed. Dr. Altstein has also discovered that in vivo the first processing endopeptidase cleaves on the carboxyl-side of the Arg in the Lys-Arg signal in the OT-prohormone. At present she is conducting studies on the kinetics of these enzymatic converting events in vivo using pulse-chase paradigms. The morphological distribution of AVP and OT development in the mouse is currently under study by Dr. A. Nieburgs, who is using immunocytochemical techniques and antibodies generated in our laboratory against these peptides. These studies will be followed by in situ hybridization histochemistry analysis using cDNA probes for AVP and OT precursor mRNAs. Dr. Y. Hara has cloned the mouse AVP and OT genes, has completed restriction maps of these genes, and has found that they are located in the mouse, as in the human, on the same chromosome separated by about 6 Kbases. Dr. Hara is currently sequencing these genes, and is focusing especially on their 5'-upstream regions to elucidate potential promotor and enhancer sequences. These sequences will be used in transfection and transgenic mouse experiments in the future to study cell-specific expression and regulation of these peptides. Dr. K. Conway has analysed the distribution and development of the related vasotocin and mesotocin peptide containing neurons (ca. 4,000) in the adult Xenopus brain, and has analyzed the cross reactivities of mammalian Np Mabs in these neurons. He has discovered a fascinating epitope switch in the frog Np versus the rat Np. His main objective to examine the lineages of the AVT and MT neurons in Xenopus is now in progress. Dr. M. H. Whitnall has been studying the organization of CRF neurons in the hypothalamus, using serial section immunocytochemical techniques especially developed in our laboratory for this purpose (by Dr. Whitnall and Ms. S. Key). Dr. Whitnall has demonstrated that: 1) The CRF neurons are composed (in normal rats) of two populations, one which contains AVP in co-existence with CRF in its secretory vesicles (50%) and one which only contains CRF (50%); 2) This co-existence phenomenon has been demonstrated by using 3 antibodies against the three components of the AVP prohormone (AVP, NP-VP, and the glycopeptide), in itself raising the ultrastructural analysis of co-existence to a new level of comprehensiveness; 3) He has shown that following adrenalectomy there is only one population, i.e., the co-existent one, thereby demonstrating a neuronal plasticity relative to function in this system. At present, the above studies which were done at the level of nerve terminals in the median eminence, are being performed also at the perikaryon level in the hypothalamus. Dr. C. Bondy has studied the control of AVP and OT secretion in the neurohypophysis. Using an in vitro stimulation paradigm, she has demonstrated the importance of potassium channels in the regulation of secretion at the nerve terminal level, and has shown that dynorphin, co-secreted with vasopressin, is the endogenous opioid peptide which selectively inhibits OT secretion presumably by affecting calcium channel opening. Finally, Dr. S. Wray has developed an in vitro organotypic culture of the LHRH system, and has succeeded in growing substantial numbers of LHRH neurons for as long as one month in vitro. Of even greater significance, she has been able to co-culture brain

stem, hypothalamus, and pituitary successfully so as to allow a study of these three-dimensional components in the brain in a two-dimensional space. In these experiments she has shown by immunocytochemical and in situ hybridization histochemical techniques that in addition to LHRH neurons, neurons synthesizing dopamine, serotonin, norepinephrine, vasoactive intestinal peptide, vasopressin, oxytocin, CRF, and gamma amino butyric acid prosper in this environment in an organotypic fashion. Together with Dr. P. Lowenstein, she has also co-cultured substantia nigra dopamine neurons together with striatum. She is currently studying a variety of regulatory mechanisms under these controlled in vitro conditions.

In addition to the above studies on peptidergic neurons, our Section has worked on macromolecular involvement in neuronal structure and recognition. Three model systems are being used for this purpose; the Xenopus developing nervous system, the squid giant axon, and the mouse hypothalamo-neurohypophysial system. Each of these systems makes a unique contribution to the study of neuronal structure and development. Dr. B. Szaro has completed his analysis of Mabs against Xenopus neuronal cytoskeleton, and is currently using these characterized Mabs to evaluate the expression of cytoskeleton elements during nervous system development. Some of these brain specific Mabs (e.g., MAP-1, neurofilaments, etc.) are recognizing specific cells and fibers in the nervous system as early as stage 33 in development, and efforts are currently under way to move to the neural plate stage. Dr. Szaro has accumulated a battery of anti-neurofilament antibodies, in part in collaboration with Ms. S. House in our laboratory, and also from Dr. V. Lee (Univ. of PA). These antibodies will be used in immunocytochemical and in blastomere injection studies in the near future, to study the effects of neurofilament protein perturbation on axonal development. Dr. L. Charnas has cloned a unique neurofilament protein cDNA from the Xenopus nervous system, and is currently sequencing this clone. Dr. H. C. Pant's studies in the squid giant axon system on neurofilament structure and function continue to support the hypothesis that the genesis of neurofilament structure is determined topographically in the neuron, i.e., biosynthesis and assembly in the cell body, phosphorylation in the axon, and proteolysis in the terminal. This topographic organization of regulation of post-translational modification is currently under study with regard to mechanism. In addition, the studies on the squid nervous system have contributed to our understanding of the significance of the autophosphorylation of Ca^{2+} /CaM protein kinase II, and on the important role of Ca^{2+} -binding proteins in axoplasm as calcium buffers. Other studies by Dr. Pant and Dr. J. Shah have been conducted on the ATP-dependent accumulation and InsP_3 -induced release of calcium by rat brain microsomes. The AVP and OT mouse genes being cloned by Dr. Hara (see above) are planned to be used in this component of the Section's work as well. We plan to make constructs of the AVP and OT gene promoters to cytoskeletal proteins (e.g., neurofilament proteins) and to study the

consequences of expression of these proteins in the AVP and OT neurons in transgenic mice.

II. Section on Enzyme Chemistry

Investigations of the relationships between structure, function and mechanism of cation transport constitute the major projects in the Section on Enzyme Chemistry. Other studies in the section relate to nerve regeneration.

A number of new strategies for investigating the structural basis of ion transport have become practicable within the past year because data on the complete amino acid sequences of the Na,K-ATPase and of several related transport ATPases have become available. We have developed a series of antibodies directed against known ATPase sequences and we are employing them as structural and functional probes of the Na,K-ATPase. Current studies are directed toward the identification of domains of the ATPase that make up the cation binding and transport sites. Isozyme-specific antibodies are also being developed and employed in an investigation of their distribution and functional significance in brain.

We are continuing studies of the transient kinetics of the phosphorylation and dephosphorylation of catalytic site of the Na,K-ATPase. The objective of the current studies is to explain the complex time course of these reactions.

A collaborative project on quantitative studies of nerve regeneration is in progress. Nerve regeneration can be logically considered in 4 aspects: 1) the peripheral signal; 2) the neuronal growth response; 3) axonal guidance; and 4) re-establishment of synapses. Current work is directed at quantitatively evaluating the neuronal growth response in peripheral nerves as reflected by the changes in neurofilament proteins. Baseline data have been acquired and experiments in progress suggest that the further analysis of neurofilament protein composition in different stages of nerve response to injury can provide a useful index of the regenerative process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-00813-26 LMC																														
PERIOD COVERED October 1, 1986 through September 30, 1987																																
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Enzymological Aspects of Neural Functions																																
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P. I. :</td> <td style="width: 45%;">R. W. Albers, Ph.D.</td> <td style="width: 20%;">Section Head</td> <td style="width: 15%;">LNC</td> <td style="width: 5%;">NINCDS</td> </tr> <tr> <td>Others :</td> <td>A. K. Hazra, Ph.D.</td> <td>Visiting Associate</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td>:</td> <td>A. S. Hobbs, Ph.D.</td> <td>Expert Consultant</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td>:</td> <td>W. T. Link, Ph.D.</td> <td>Staff Fellow</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td>:</td> <td>P. M. Rowe, Ph.D.</td> <td>Staff Fellow</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td>:</td> <td>E. Summers</td> <td>Technician</td> <td>LNC</td> <td>NINCDS</td> </tr> </table>			P. I. :	R. W. Albers, Ph.D.	Section Head	LNC	NINCDS	Others :	A. K. Hazra, Ph.D.	Visiting Associate	LNC	NINCDS	:	A. S. Hobbs, Ph.D.	Expert Consultant	LNC	NINCDS	:	W. T. Link, Ph.D.	Staff Fellow	LNC	NINCDS	:	P. M. Rowe, Ph.D.	Staff Fellow	LNC	NINCDS	:	E. Summers	Technician	LNC	NINCDS
P. I. :	R. W. Albers, Ph.D.	Section Head	LNC	NINCDS																												
Others :	A. K. Hazra, Ph.D.	Visiting Associate	LNC	NINCDS																												
:	A. S. Hobbs, Ph.D.	Expert Consultant	LNC	NINCDS																												
:	W. T. Link, Ph.D.	Staff Fellow	LNC	NINCDS																												
:	P. M. Rowe, Ph.D.	Staff Fellow	LNC	NINCDS																												
:	E. Summers	Technician	LNC	NINCDS																												
COOPERATING UNITS (if any) J. P. Froehlich, NIA, NIH, Baltimore, MD R. H. Huang, U. Mo. Health Sci., Kansas City, MO																																
LAB/BRANCH Laboratory of Neurochemistry, DIR, NINCDS																																
SECTION Section on Enzyme Chemistry																																
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																																
TOTAL MAN-YEARS 5.5	PROFESSIONAL 4.75	OTHER .75																														
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																																
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided) This project is comprised of research into the structure and functioning of <u>ion transport systems</u>. There are 3 subprojects. 1) Research into the relation between the structure and the functioning of the sodium pump. A series of antibodies directed to selected portions of the primary structure of the <u>transport ATPase</u> are being used as probes into these relationships. 2) The significance of the existence of isozymes of the sodium pump ATPase is under investigation utilizing techniques for their physical separation and employing antibodies directed against differentiating portions of their primary sequences to study their cytochemical localizations. 3) Rapid kinetic techniques are being applied to an investigation of the multiphasic time course of phosphorylation and dephosphorylation of the sodium pump protein during its catalytic cycle.																																

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02701-02 LNC
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Quantitative Studies of Nerve Regeneration		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P. I. : R. W. Albers, Ph.D. Others : E. Summers	Section Head Technician	LNC LNC NINCDS NINCDS
COOPERATING UNITS (if any) L. Guth, University of Maryland School of Medicine		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Section on Enzyme Chemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.5	PROFESSIONAL .25	OTHER .25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This is a collaborative project with the aim of developing, validating and applying a quantitative measure of nerve regeneration to the study of factors that promote or regulate the rate and extent of axonal outgrowth in both peripheral and central nervous tissues. We have developed a quantitative fluorescent method based on immunochemical detection of phosphorylated neurofilament protein. The technique has been validated by applying it to a study of sciatic nerve degeneration and regeneration subsequent to nerve crush. Studies are in progress to extend this technique to studies of spinal cord degeneration and regeneration subsequent to controlled crush injury.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02723-01 LNC *
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Peptides in the Adult and Developing Vertebrate Nervous Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P. I. : Harold Gainer, Ph.D. Chief LNC NINCDS Others : Mark Whitnall, Ph.D. Senior Staff Fellow LNC NINCDS : Kevin Conway, Ph.D. Staff Fellow LNC NINCDS : Susan Wray, Ph.D. PRAT Fellow LNC NINCDS : Miriam Altstein, Ph.D. Visiting Fellow LNC NINCDS : Sharon Key, B.S. Biologist LNC NINCDS : Pedro Lowenstein, M.D. Visiting Fellow LNC NINCDS : Alexander Wheaton Biologist LNC NINCDS		
COOPERATING UNITS (if any) Dr. B. Gahwiler, Sandoz Ltd., Switzerland; Dr. D. Smyth, MRC, London; M. Castel, Hebrew University; Dr. Carolyn Bondy, NICHD; Dr. Tom Zoeller, LCB, NIMH		
LAB/BRANCH Laboratory of Neurochemistry, DIR, NINCDS		
SECTION Section on Cellular and Developmental Neurobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland		
TOTAL MAN-YEARS 4.4	PROFESSIONAL 2.4	OTHER 2.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies of <u>peptide coexistence</u> have revealed two <u>CRF neuron</u> sub-types in the <u>hypothalamus</u> (PVN), CRF/AVP⁺ and CRF⁺/AVP⁻ with respect to coexistence of these two peptides. Each subtype represents about half of the total CRF⁺ neuronal population in the PVN and of the total CRF⁺ neuronal population in the PVN and of the total CRF⁺ axonal population in the <u>median eminence</u>. The existence of these subtypes can account for the variations in CRF to AVP ratios in portal blood with different forms of stress. Analysis of the <u>development</u> of <u>vasopressin</u> and <u>oxytocin neurons</u> by immunocytochemical and RIA procedures have shown that there is a profound differential expression of peptides during <u>fetal development</u>. The vasopressin (AVP) neurons are more precocious than oxytocin (OT) neurons during development in extent of peptide expression, and in efficiency and extent of <u>precursor processing</u>, so that a stable intermediate form of oxytocin (OT-Gly₁₀-Lys₁₁-Arg₁₂) is found during embryonic life. Vasotocin (AVT) and mesotocin (MT) neurons have been mapped in 3-dimensions in adult <u>Xenopus</u> hypothalamus, and neurophysin immunoreactivities have been documented. Expression of these peptides appears at least at stage 45 in <u>Xenopus</u> development. An <u>organotypic tissue culture model</u> of rat LHRH neurons has been developed, and preliminary data suggests <u>trophic interactions</u> between brain stem, hypothalamus, and pituitary regions <u>in vitro</u> studies on the <u>opioid peptide</u> sensitivity of AVP and OT secretion from the neural lobe <u>in vitro</u> have shown that OT secretion is inhibited by an endogenous opioid which appears to be secreted from the VP terminals, and also by exogenous administered <u>dynorphin</u>. *This project has been transferred from the LNN, DIR, NICHD (formerly Z01-HD-00058-11 LNN) to the LNC, DIR, NINCDS.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02724-01 LNC [*]																									
PERIOD COVERED October 1, 1986 to September 30, 1987																											
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Molecular Mechanisms in Neuronal Structure and Function																											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P. I. :</td> <td style="width: 40%;">Harold Cainer, Ph.D.</td> <td style="width: 20%;">Chief</td> <td style="width: 15%;">LNC</td> <td style="width: 10%;">NINCDS</td> </tr> <tr> <td>Others :</td> <td>Ben Szaro, Ph.D.</td> <td>Staff Fellow</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Shirley House</td> <td>Biologist</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Yoshinobu Hara, Ph.D.</td> <td>Visiting Fellow</td> <td>LNC</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Andra Nieburgs, Ph.D.</td> <td>NIH Extramural Fellow</td> <td>LNC</td> <td>NINCDS</td> </tr> </table>			P. I. :	Harold Cainer, Ph.D.	Chief	LNC	NINCDS	Others :	Ben Szaro, Ph.D.	Staff Fellow	LNC	NINCDS		Shirley House	Biologist	LNC	NINCDS		Yoshinobu Hara, Ph.D.	Visiting Fellow	LNC	NINCDS		Andra Nieburgs, Ph.D.	NIH Extramural Fellow	LNC	NINCDS
P. I. :	Harold Cainer, Ph.D.	Chief	LNC	NINCDS																							
Others :	Ben Szaro, Ph.D.	Staff Fellow	LNC	NINCDS																							
	Shirley House	Biologist	LNC	NINCDS																							
	Yoshinobu Hara, Ph.D.	Visiting Fellow	LNC	NINCDS																							
	Andra Nieburgs, Ph.D.	NIH Extramural Fellow	LNC	NINCDS																							
COOPERATING UNITS (if any) L. Charnas, M.D., NICHD																											
LAB/BRANCH Laboratory of Neurochemistry, DIR, NINCDS																											
SECTION Section on Cellular and Developmental Neurobiology																											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland																											
TOTAL MAN-YEARS 2.4	PROFESSIONAL 1.4	OTHER 1.0																									
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																											
SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided) Thirteen <u>monoclonal antibodies</u> directed against adult <u>Xenopus neural cytoskeleton</u> have been generated and characterized by Western blots and immunocytochemistry. These antibodies have been used in evaluating the expression of various <u>intermediate filamen proteins</u> (including <u>neurofilament proteins</u>) in the developing <u>Xenopus</u> nervous system. A <u>Xenopus</u> brain <u>cDNA</u> library has been probed, using mouse intermediate filament protein cDNAs, for <u>intermediate filament clones</u>. Four stable clones have been isolated, and one clone apparently coding for a 57KDa neurofilament protein, is currently being sequenced. A mouse genomic library has been probed for <u>AV and OT genes</u>, using human cDNA probes. The mouse AVP and OT genes have been isolated, and are currently undergoing sequencing. *This project has been transferred from the LNN, DIR, NICHD (formerly Z01-HD-01200-01-LNN) to the LNC, DIR, NINCDS.																											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02725-01 LNC
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Calcium Metabolism and Protein Phosphorylation in Neuronal Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P. I. :	Harish C. Pant, Ph.D.	Resident Chemist LNC NINCDS
:	H. Gainer, Ph.D.	Chief LNC NINCDS
:	J. Shaw, Ph.D.	Visiting Fellow LNC NINCDS
COOPERATING UNITS (if any) Dr. Claude Klee, NCI; Dr. P. E. Gallant, LN, NINCDS; Dr. Tom Soderling, Vanderbilt University; Dr. R. Cohen, University of Illinois		
LAB/BRANCH Laboratory of Neurochemistry, DIR, NINCDS		
SECTION Section on Cellular and Developmental Neurobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.2	0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Analyses of <u>cytoskeletal proteins</u> in the <u>squid nervous system</u> has been facilitated by the production of a panel of <u>monoclonal antibodies</u> (Mabs) against these proteins. In the squid, these Mabs have been used to show that <u>phosphorylation of neurofilaments</u> occurs only in the <u>axon</u>. Analysis of the <u>protein kinase-calcium activated protease-neurofilament</u> complex in the squid giant axon has shown that the protein kinase is similar to type II casein kinase, and that an inhibitor of this kinase is located in the cell bodies in the stellate ganglion. The protease is found throughout the cell, except at the terminals, where it appears to undergo a cycle of activation and inactivation. Studies of <u>calcium-binding proteins</u> in axoplasm have revealed large quantities of <u>calmodulin</u> and <u>calneurin</u>, a calcium-dependent <u>protein phosphatase</u>. Squid optic lobe synaptosomes contain abundant <u>calcium/calmodulin-dependent protein kinase II</u> which undergoes <u>autophosphorylation</u>, which leads to calcium-independent activity. ATP dependent accumulation of calcium was observed in <u>rat brain microsomes</u> and <u>InsP₃</u> caused a rapid release of calcium which was followed by a slow re-uptake. The two organelles thought to be responsible for calcium storage are endoplasmic reticulum and mitochondria. We found that InsP₃- induced Ca⁺⁺ release was not observed in mitochondria. Moreover, the presence of mitochondrial inhibitors, oligomycin and sodium azide, during calcium uptake, did not affect the ability of InsP₃ to release Ca⁺⁺ from the microsomes. In addition, electron microscopy did not reveal mitochondria in the microsomal fraction. Brain microsome may provide a model for investigating the regulation of Ca⁺⁺ mobilization in the central nervous system.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02254-11 LNC
PERIOD COVERED October 1, 1986 through September 30, 1987 *		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Repair of Injured Nervous Tissue with Foreign Grafts		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) P. I. : A. A. Zalewski, M.D. Section Head LNC NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurochemistry, DIR, NINCDS		
SECTION Section on Neuronal Development and Regeneration		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0	PROFESSIONAL 0	OTHER 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) * This project has been transferred to the LNCGR, DIR, NINCDS (on 10/86).		

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neuro-otolaryngology

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987
Laboratory of Neuro-Otolaryngology, DIR
National Institute of Neurological and
Communicative Disorders and Stroke

Jorgen Fex, M.D., Ph.D, Chief

The Laboratory has continued to provide new knowledge within the framework of its two Projects: Project Number Z01 NS 02216-12 LNO, Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis and Project Number Z01 NS 02217-12 LNO, Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus. We contribute towards a better understanding of how the inner ear translates sound waves into nerve activity and how the cochlear nucleus processes the auditory information that it receives from the inner ear.

We are continuing to carry out experiments on isolated hair cells, seeking to establish the structural basis and mechanism of such electrically evoked motile responses that we reported in last year's Annual Report. We have built a photodiode based instrument that will permit continuous monitoring of very high frequency movements in hair cells. We are initiating studies to characterize membrane properties of the hair cells by patch clamping. We have improved our method of isolating hair cells from guinea pig cochlea and we are trying to obtain isolated hair cells from vestibular organs that display giant stereocilia. Such stereocilia may permit patch clamping of stereociliar membrane domains presumed to contain the mechano-electrical transducer receptors. We are studying the structure of hair cells stereocilia and their interactions with the tectorial membrane in the organ of Corti and the gelatinous membrane and otoconial crystals in otolith organs, using conventional and cryogenic techniques for electron microscopy. We have determined the organization of an extracellular filamentous matrix at the surface of frog vestibular organ which might be involved in securing the otoconial membrane and in modulating sensitivity of the hair bundles. We are also carrying out studies involving immunocytochemical characterization of both cytoskeletal and extracellular matrix elements in these tissues.

Several studies aimed at understanding basic mechanisms of intracellular transport and of cell motility are in progress. Ultrastructural studies of organelle translocation have revealed an extensive interface of interaction between organelle and microtubule, the presence of defined cross-bridge elements, and a tight binding implied by the deformation of organelles and microtubules during translocation. A microtubule translocator protein in *Acanthamoeba* has been purified that possesses a very high microtubule-activated Mg^{2+} ATPase activity and that has the ability to translocate latex beads at high rate from the minus-end to the plus-end of purified brain microtubules. We have obtained evidence that the integrated cytoplasmic streaming in *Characean* giant cells depends on the shearing force generated along stationary actin cables by an ATPase associated with the adjacent surfaces of an endoplasmic reticulum network.

We are continuing our search for useful markers of membranes and other components of different cell populations in auditory sensory organs. We are carrying out immunocytochemical experiments towards this aim, using anti-ACh-receptor antibodies and other antibodies. We have recently found tyrosine

hydroxylase-like immunoreactivity in putative catecholaminergic efferent nerve fibers in the guinea pig organ of Corti; these results are in print. We have now also seen that antibodies to prostaglandin D₂ synthetase from rat brain evoke immunoreactivity in cell populations of the adult rat cochlea, including inner and outer hair cells.

As mentioned in last year's Annual Report, we initiated a collaboration with Dr. J. T. Corwin, for studies aiming at an understanding of how the ever continuing growth of the hearing organ of shark, fish and toad is programmed and how it may be controlled. Therefore, as part of such studies, at the very end of FY 1986, we took the first steps towards producing monoclonal antibodies against antigenic material from shark auditory organs. For many reasons, our collaboration, including this particular study, proved to be impractical and was terminated. As one of several other approaches towards a long range aim of the LNO to analyse and describe the auditory sensory organ also in terms of molecular biology, we have significantly extended our collaboration with the Laboratory of Molecular Biology (LMB), DIR, NINCDS. Through this collaboration, staff members of the LNO are becoming increasingly familiar with the applicability of techniques of molecular biology. We expect eventually to use these techniques in the LNO for studying specific characteristics of the organ of hearing. Presently, our collaborative study, together with the LMB, concerns the molecular biology of the genes involved in the regulation and synthesis of the enzymes glutamate dehydrogenase and glutaminase. In collaboration, a cDNA molecule has been isolated and characterized, showing that this cDNA encoded the partial sequence of the enzyme glutamate dehydrogenase; these results are in print. Also, a rat expression library has been screened with antibodies against glutaminase. A glutaminase cDNA was isolated and characterized. The goal of our current research is to identify cDNA molecules which encode the complete sequence of glutamate dehydrogenase and to identify and sequence cDNAs to obtain a complete sequence for glutaminase. Both these enzymes are closely associated with the metabolism of the neurotransmitter glutamate, a candidate for neurotransmission at auditory nerve synapses.

We are continuing our multifaceted project that provides new knowledge on the identification and characterization of neurotransmitters, neuromodulators and related enzymes and receptors active at major synapses in brainstem auditory nuclei. This includes studying the effects of lesions in the cochlea on auditory nerve and cochlear nucleus.

Using specific antibodies against GABA and glycine, putative GABAergic and glycinergic neurons in auditory nuclei in guinea pig were identified with immunocytochemistry. The general findings on the distributions of GABA, glycine and the glycine receptor were presented in the Annual Reports of the last two years. We have continued to study the co-localization of GABA and glycine in a group of neurons in the dorsal cochlear nucleus using double labeling techniques. Three populations of neurons were defined in the dorsal cochlear nucleus, those containing only GABA, those containing only glycine, and those containing both GABA and glycine. We found a similar phenomenon in the cerebellum where the Golgi cells were the only GABA-containing cell population to also contain glycine.

We are also determining if any GABA or GAD containing presynaptic terminals in the cochlear nucleus are associated with postsynaptic glycine receptor immunoreactivity. Preliminary studies indicate this is the case at some synapses in

the dorsal and posteroventral cochlear nuclei, but synapses are also found containing only glycine receptor immunoreactivity or only GABA or GAD immunoreactivity. Therefore, the cochlear nucleus differs from the spinal cord where it is reported that all GAD containing presynaptic terminals have postsynaptic glycine receptor immunoreactivity.

The sources and terminations of GABA-containing and glycine-containing neurons in auditory nuclei are being studied using retrograde transport of HRP combined with immunocytochemistry. It was found that the large glycine-immunoreactive neurons scattered throughout the ventral cochlear nucleus project to the contralateral cochlear nucleus. Preliminary findings also indicate that the small glycine-immunoreactive neurons in the deep layers of the dorsal cochlear nucleus project to the anteroventral cochlear nucleus.

Monoclonal antibodies against neurofilament subunits were used in immunocytochemical studies of the auditory nerve and spiral ganglion of guinea pig. Type I and Type II spiral ganglion cells differed in the amounts of neurofilament subunits as determined by antibody binding. With experimentally induced hair cell loss, changes in the expressions of neurofilament subunits were apparent in type I spiral ganglion cells.

During this past year we have continued and expanded a project to biochemically characterize excitatory amino acid receptors. We have concentrated on solubilizing and biochemically characterizing a kainic acid binding component from brain. Kainic acid receptors were solubilized from rat and frog brain. The soluble receptors contained high and low affinity components and were pharmacologically similar to the membrane-associated receptors. The relative abundance of kainic acid receptors in frog brain makes this a preferred source of material for the purification of these receptors. Significant purification (up to 40 fold) can be obtained through chromatography on DEAE Sepharose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02216-12 LNO																								
PERIOD COVERED October 1, 1986 to September 30, 1987																										
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Jörgen Fex, M.D., Ph.D.</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">LNO, DIR, NINCDS</td> </tr> <tr> <td rowspan="5">OTHERS:</td> <td>B. Kachar, M.D.</td> <td>Visiting Scientist</td> <td>LNO, DIR, NINCDS</td> </tr> <tr> <td>M. E. Schneider, Ph.D.</td> <td>Staff Fellow</td> <td>LNO, DIR, NINCDS</td> </tr> <tr> <td>M. Kowalska, M.D., Ph.D.</td> <td>Visiting Fellow</td> <td>LNO, DIR, NINCDS</td> </tr> <tr> <td>M. J. Frye</td> <td>Electronics Technician</td> <td>LNO, DIR, NINCDS</td> </tr> <tr> <td>S. A. Kranwinkel, B.S.</td> <td>Chemist</td> <td>LNO, DIR, NINCDS</td> </tr> <tr> <td></td> <td>M. H. Parakkal</td> <td>Biol. Lab. Technician</td> <td>LNO, DIR, NINCDS</td> </tr> </table>			PI:	Jörgen Fex, M.D., Ph.D.	Chief	LNO, DIR, NINCDS	OTHERS:	B. Kachar, M.D.	Visiting Scientist	LNO, DIR, NINCDS	M. E. Schneider, Ph.D.	Staff Fellow	LNO, DIR, NINCDS	M. Kowalska, M.D., Ph.D.	Visiting Fellow	LNO, DIR, NINCDS	M. J. Frye	Electronics Technician	LNO, DIR, NINCDS	S. A. Kranwinkel, B.S.	Chemist	LNO, DIR, NINCDS		M. H. Parakkal	Biol. Lab. Technician	LNO, DIR, NINCDS
PI:	Jörgen Fex, M.D., Ph.D.	Chief	LNO, DIR, NINCDS																							
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	S. A. Kranwinkel, B.S.	Chemist	LNO, DIR, NINCDS																							
	M. H. Parakkal	Biol. Lab. Technician	LNO, DIR, NINCDS																							
COOPERATING UNITS (if any) Lab. of Biophysics, DIR, NINCDS (K. Iwasa); Lab. of Molecular Biology, DIR, NINCDS (C. Banner and Y. Nakatani); Lab. of Neurobiology, DIR, NINCDS (T. S. Reese); Lab. of Cell Biology, NHLBI (E. D. Korn); Kresge Hearing Research Institute, Univ. of Michigan, Ann Arbor, MI (R. A. Altschuler); *(Continued)																										
LAB/BRANCH Laboratory of Neuro-otolaryngology																										
SECTION																										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																										
TOTAL MAN-YEARS: 5.3	PROFESSIONAL: 3.6	OTHER: 1.7																								
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This multifaceted project provides new knowledge of <u>auditory mechanisms</u> through studies of <u>auditory sensory organs</u>, using <u>small mammals</u>, and <u>bullfrogs</u>. <u>Immunocytochemical studies of receptors and other markers in the cochlea</u> are being continued. We have found <u>tyrosine hydroxylase-like immunoreactivity</u> in putative catecholaminergic efferent nerve fibers in the guinea pig organ of Corti. Also, antibodies to <u>prostaglandin D2 synthetase</u> from rat brain were found to evoke immunoreactivity in cell populations of the adult rat cochlea, including inner and outer hair cells. We are continuing our studies of <u>motile mechanisms</u> in auditory sensory cells. We have determined the organization of an <u>extracellular filamentous matrix</u> at the surface of the frog vestibular organ which might be involved in securing the otoconial membrane and in modulating sensitivity of the hair bundles. Several studies of basic <u>intracellular transport and cell motility mechanisms</u> are in progress. Ultrastructural studies have revealed an extensive interface of interaction between organelle and microtubule, cross-bridge elements, and a tight binding implied by the deformation of organelles and microtubules during <u>organelle translocation</u>. An <u>Acanthamoeba microtubule translocator protein</u> has been purified that possesses a very high microtubule-activated Mg-ATPase activity. We have evidence that the integrated cytoplasmic streaming in <u>Characean</u> giant cells depends on the shearing force generated along stationary actin cables by an ATPase associated with the adjacent surfaces of an endoplasmic reticulum network. In collaboration with the Laboratory of Molecular Biology, DIR, NINCDS we are continuing a study of <u>genes involved in the regulation and synthesis of the neurotransmitter glutamate</u>. Presently, this study aims at identifying <u>cDNA</u> molecules that <u>encode</u> the complete sequence of <u>glutamate dehydrogenase</u> and at identifying and sequencing cDNAs to obtain a complete sequence for <u>glutaminase</u>.																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02217-12 LNO
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Robert J. Wenthold, Ph.D. Chemist LNO, DIR, NINCDS OTHERS: D. R. Hampson, Ph.D. Staff Fellow LNO, DIR, NINCDS J. Dau, Ph.D. Visiting Fellow LNO, DIR, NINCDS M. J. Frye Electronics Technician LNO, DIR, NINCDS D. Huie, B.S. Chemist LNO, DIR, NINCDS M. H. Parakkal Biol. Lab. Technician LNO, DIR, NINCDS K. A. Reeks, B.S. Biologist LNO, DIR, NINCDS		
COOPERATING UNITS (if any) Laboratory of Molecular Biology, DIR, NINCDS (C. Banner); NEI, NIH (M.D. Oberdorfer); Department of Anatomy, University of Conn. Health Center (R.L. Saint Marie and D.K. Morest); Department of Otolaryngology, Medical Univ. of South Carolina, Charleston, SC (J.C. Adams); *(Continued)		
LAB/BRANCH Laboratory of Neuro-otolaryngology		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 4.2	PROFESSIONAL 3.0	OTHER 1.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) This multifaceted project provides new knowledge on the identification and characterization of neurotransmitters, neuromodulators and related enzymes and receptors active at major synapses in brainstem auditory nuclei. The effects of lesions in the cochlea on auditory nerve and cochlear nucleus are determined. Using specific <u>antibodies</u> against GABA and <u>glycine</u>, putative GABAergic and glycinergic neurons in auditory nuclei in guinea pig were identified with <u>immunocytochemistry</u>. In the <u>dorsal cochlear nucleus</u>, <u>double labeling</u> studies showed the presence of neurons containing GABA, neurons containing glycine and neurons containing both GABA and glycine. The <u>coexistence</u> of GABA and glycine immunoreactivities in a population of cell bodies and terminals indicates that both amino acids may be released from these neurons. Projections of glycine- and GABA-containing neurons were determined using immunocytochemistry and retrograde transport of horseradish peroxidase. Monoclonal antibodies against <u>neurofilament subunits</u> were used in immunocytochemical studies of the auditory nerve and spiral ganglion of guinea pig. Type I and Type II <u>spiral ganglion cells</u> differed in the amounts of neurofilament subunits as determined by antibody binding. With experimentally induced hair cell loss, changes in the expressions of neurofilament subunits were apparent in Type I spiral ganglion cells. We have continued our characterization of the neurotransmitter, <u>glutamate</u>, by studying its <u>receptors</u> and related enzymes. In collaboration with the Laboratory of Molecular Biology, NINCDS, a <u>rat expression library</u> has been screened with antibodies against glutaminase. A <u>glutaminase cDNA</u> was isolated and characterized. <u>Kainic acid receptors</u> were solubilized from rat and frog brain. The <u>soluble receptors</u> contained high and low affinity components and were pharmacologically similar to the membrane-associated receptors. Ion exchange chromatography was used to purify the frog brain receptor.		

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neuropathology and Neuroanatomical Sciences
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987
Laboratory of Neuropathology and Neuroanatomical Sciences, DIR
National Institute of Neurological and Communicative
Disorders and Stroke

Igor Klatzo, M.D. Chief

The Laboratory of Neuropathology and Neuroanatomical Sciences has further pursued its general program focused primarily on elucidation of pathophysiology of brain edema and cerebral ischemia, which is the main pathological substrate of stroke. In view of the important role cerebrovascular permeability changes play in these conditions, special attention was given to studies on the behavior of the blood-brain barrier. Our overall motivation and aim in these studies remains the notion that, by exploring the nature of pathomechanisms involved in cerebral ischemia and edema, it will be possible to design proper therapeutic methods for treatment of stroke and related conditions.

The Section of Cerebrovascular Pathology has been concerned primarily with the evaluation of the effects of post-ischemic circulatory disturbances on degree of an ischemic brain injury and with further observations on ischemic penumbra, a condition in which, in spite of ischemic damage, the neurons still retain ability for recovery.

An experimental condition in which both these aspects of post-ischemic injury could be studied involved application of our newly-developed experimental model for repetitive ischemic insults in gerbil. This model, which provides an easy and reproducible way for systematic study of repeated ischemic insults of desired frequency and duration, requires minimal anaesthesia being virtually free of trauma and pain, the occluding device being implanted at least 7 days prior to experimentation. Our observations on repeated three 5 minute bilateral occlusions in gerbils revealed a striking cumulative effect of three occlusions on development of edema and degree of damage to the brain parenchyma, when the insults were spaced at the period of post-ischemic hypoperfusion. This close relationship between the brain injury and secondary hypoperfusion was evident from the observations showing that maximal edema and tissue injury were associated with insults which were spaced one hour apart, i.e., at the time of most pronounced hypoperfusion, whereas no cumulative effect concerning edema and tissue injury was

observed when the occlusions were 3 minutes apart or at longer than 6 hour periods. The nature of the cumulative effect and of relationship between brain injury and post-ischemic hypoperfusion was further elucidated by assays on oxygen content changes in animals subjected to 3 occlusions at 1 hour interval. The polarographic determinations of oxygen tension with plantinized microelectrodes indicated a development of secondary hypoxia which was becoming progressively more intense after each consecutive ischemic insult. The case of this secondary hypoxia appears to be an interference with microcirculation, which was brought out in series of observations on blood filling of the microcirculatory channels during and after 3 x 5 minute occlusions. These observations were carried out by using intravenous injection of Evans Blue tracer, which is recognizable by its intense red fluorescence outlining vascular channels, when frozen sections of the brain are examined under the fluorescence microscope. Our observations revealed that following recirculation from the first insult, there was a progressive reduction in the number of tracer-filled capillaries, which appeared to be compensated by progressive dilatation of larger channels, especially of arteriolar caliber.

Besides the hypoperfusion, studies in collaboration with the Section on Cerebrovascular Pathophysiology have clearly established a deleterious effect of the reactive hyperemia, which promptly follows recirculation of previously ischemic regions. It was shown in our previous studies that reactive hyperemia, rapidly following a release of arterial occlusion, is associated with the opening of the blood-brain barrier to proteins, and this intensifies development of the vasogenic brain edema and aggravates ischemic injury to the brain tissue. This mechanism has been strongly supported by further study demonstrating that reduction of the reactive hyperemia by aminophylline has a clear, beneficial effect, significantly reducing the severity of edema and brain tissue injury. Application of aminophylline may have a practical therapeutic value in neurosurgical procedures in which prevention of reactive hyperemia may be indicated.

Our studies on ischemic penumbra have been pursued under experimental conditions in which the animals are subjected to cerebral ischemia at the threshold levels, resulting in ischemic injury of brain tissue evident in various parameters, which however, show a tendency towards recovery. As experimental model served 20 minute occlusion of the middle cerebral artery in cat. Morphological changes concerning ischemic brain damage, as well as changes in regional cerebral blood flow (rCBF) and brain tissue water content by specific gravity (SG) measurements were evaluated at different periods up to 2 weeks after recirculation. Our studies revealed that in spite of being very similar in intensity and duration ischemia, both in the cerebral cortex and the caudate, the latter showed significantly higher reactive hyperemia and accumulation of water than the cerebral cortex.

Neither structure showed edema when examined after 2 weeks. Morphologically after 1 and 3 days in all animals more severe ischemic changes were observed in the caudate than the cortex. Cats sacrificed at 2 weeks showed only in 2 out of 8 animals very few focal ischemic loss of cells, otherwise the neurons appeared normal. This study demonstrates that an ischemic insult at threshold intensity may result in edema and ischemic brain injury which are fully reversible and that, under the equal in intensity ischemia, the caudate appears to be more susceptible to development of reactive hyperemia and brain edema. Our other studies on ischemic penumbra are focused on establishing the threshold for ischemic injury in gerbils and in ascertaining whether repeated, very short insults may produce recognizable ischemic lesions.

In addition, the Section on Cerebrovascular Pathology has extended research, under Dr. Nowak's direction, to investigating molecular events following transient ischemia, namely induction of stress proteins and changes in opioid peptides. Otherwise, the methodological studies have focused on comparisons of hydrogen clearance with other methods used in measurements of the cerebral blood flow.

Studies of molecular events in brain following transient ischemia have grown out of an initial interest in mechanisms responsible for the striking reductions in brain protein synthesis persisting for many hours following recirculation. While basic questions of protein synthesis regulation continue to be investigated two other more specific projects have evolved.

First, induction of the major mammalian stress protein, hsp70, have been extended to the immunocytochemical level and reveal a striking and reproducible anatomical and temporal distribution pattern. Hsp70 immunoreactivity appears in neurons of hippocampus and other brain regions during several days following recirculation, being notably induced in dentate granule cells and CA3 neurons which survive the insult, but not in CA1 neurons which fail to survive. Future studies will focus on levels and distribution of hsp70-related mRNA sequences, with an interest in evaluating the role of stress proteins in cell survival and cell death following ischemia.

A second area of interest involves opioid peptides derived from prodynorphin and their physiology and pathology following ischemia. Dynorphin A, present in hippocampal dentate granule cells, has been shown to be persistently decreased following transient ischemia, and preliminary results suggest reductions in prodynorphin mRNA as well. Further studies will evaluate more directly the localization of these changes using immunocytochemistry and in situ hybridization techniques. These studies

provide basic information on changes in opioid peptide systems following ischemia, against which ongoing clinical trials of opiate antagonists in stroke treatment may be compared.

Finally, an important methodological issue which remains to be resolved centers around the large discrepancies frequently obtained in blood flow measurements using hydrogen clearance electrodes vs. other tracer diffusion methods. Our results have largely clarified this issue in the gerbil, and demonstrate the importance of using chronically implanted electrodes in order to obtain valid measurements with hydrogen clearance. These studies are currently being extended to the cat.

The Section on Cerebrovascular Pathophysiology has been concentrating its efforts on the study of post-ischemic events by correlation of various physiological parameters in order to elucidate the nature of various pathomechanisms involved.

One of the main foci in these studies has been a study of oxygen tension in the brain cortex parenchyma and its relationship to cerebral blood flow before, during and following single and repetitive ischemic attacks. Penetrating polarographic microelectrodes were used to sample and follow continuously pO_2 levels in the parietal cortex, of lightly anesthetized, stereotactically restrained Mongolian gerbils. It was expected that oxygen tensions would correlate well with the cerebral blood flow levels in the same regions. However, important and different features became manifest early in these studies. One was that the measured oxygen tensions varied widely, tensions as low as zero to more than 50 tor were found from place to place and at different depths with little or no correlation with depth or locality. Remarkable constancy of value for a particular place or depth was present when re-examined. When this data was displayed as a bar histogram showing frequency of particular levels of pO_2 , the distribution was found to be gaussian shaped with its mean bar at the 20 to 25 tor interval. Transient cerebral ischemias were produced by temporary bilateral occlusion of both carotid arteries for variable durations and repetitions after a suitable interval. These ischemias produced a prompt drop in the pO_2 to negligible levels within a few seconds. Recirculation after release of the occlusion was associated with recovery of the pO_2 promptly if the duration of ischemia was less than about 1 minute usually with an overshoot of the pO_2 with respect to the pre-ischemic control values. With longer durations of ischemia, the recovery of the pO_2 was increasingly sluggish. At five minutes of ischemia, the delay in appearance of a pO_2 could be of the same duration. Usually, in contrast to short duration ischemias, there would be little or no overshoot of the pO_2 . Distribution histograms of pO_2 values such as the one previously mentioned were made following the five minute occlusion.

This showed a shift of the mean pO_2 values towards low values. Periodically, over a period of some hours following a five minute occlusion, distribution histograms, were constructed. These showed progressive shift with time of the pO_2 values to higher values. At the end of 24 hours, the distribution resembled the pre-ischemic distribution.

The other studies on pathophysiological aspects of cerebral ischemia were concerned with elucidation of mechanisms involved in reactive hyperemia.

Reactive hyperemia is an abnormal increase in regional cerebral blood flow (rCBF) immediately following the release of a temporary occlusion of a major artery supplying blood to a region of the brain.

Previous studies using hydrogen clearance method as a means of estimating cerebral blood flow indicated a hyperemic phase following a transient ischemia of 5 minutes in the cortex in gerbils. This was not seen in pO_2 levels. This discrepancy was examined using Laser doppler velocimetry - a relatively new technique which can be applied to the cortex. These experiments clearly showed a hyperemia-like phase following release of the occlusion of both carotids for 5 minutes. However, the maximum value of indicated blood flow did not exceed the pre-ischemic value. Following this peak, the Laser doppler indicated blood flow slowly and steadily dropped to a fraction of the pre-ischemic values. These results once again did not correlate exactly with the pO_2 levels nor with the hydrogen clearance evidence of frank hyperemia. Furthermore intensive investigation of these phenomena will be necessary to obtain an insight into the mechanism of cerebrovascular events in repetitive transient ischemias of the brain.

In collaboration with the Section on Cerebrovascular Pathology, aminophylline, the most widely used broncho dilator drug was tried in a series of cats subjected to 1 hour MCA occlusion. Cerebral blood flow was monitored in the cortex and in the caudate nucleus. The presence of edema was determined gravimetrically 3 hours after release of the occlusion and the extent of brain damage was examined microscopically 3 days and 2 weeks afterwards.

Aminophylline was successful in preventing post-ischemic hyperemia. During the post-ischemic phase, the mean rCBF in aminophyllin treated animals was 47.4% of the mean CBF in the control animals. There was no significant difference in rCBF between the two groups before or during occlusion. The edema was significantly less in the aminophylline treated animals compared with the untreated controls. Morphological evaluation showed markedly reduced brain tissue damage in the aminophylline treated animals.

The continuous goals of the Section on Neurocytobiology have been:

I) to develop and utilize new model systems for the investigation of basic mechanisms operative on the level of normal and pathologically altered blood-brain barrier (BBB) and cerebral blood flow (CBF); II) to study the metabolic processes occurring in cerebral ischemia and ischemic edema, especially their prevention and therapy.

I. During the last years, both the separately cultured endothelial and smooth muscle cells derived from dissociated cerebral microvessels have been very useful models for the continuous studies of cerebrovascular function related to the BBB, CBF and systemic blood pressure.

Three different aspects related to the function of cerebral microvessels have been investigated in the in vitro models using pure cerebrovascular endothelial and/or smooth muscle cell culture: a) the role of free fatty acids on the cellular permeability, b) effects of vasoactive substances on cerebromicrovascular carbohydrate metabolism, and c) interaction between cerebral capillary endothelium and immune cells.

a) Recently we adopted a new in vitro system consisting of cerebromicrovascular endothelium grown on dextran microcarriers to study their properties related to the function of BBB under controlled conditions. This method utilizes the fact that cell covered beads prevent accumulation of dyes like trypan blue (TB) in the microcarriers. Any increase in cellular permeability to TB results in reduced level of TB in the supernatant since TB that passes through the cellular layer binds tightly to the dextran beads.

The aim of the present investigation has been to use this model for evaluating the effects of free fatty acids which have been implicated in attenuating the blood-brain barrier permeability under pathological conditions on the permeability of separately cultured endothelial and smooth muscle cells derived from cerebral microvessels.

Both arachidonic or linoleic acid (unsaturated fatty acid) increased the TB permeability of endothelium and smooth muscle cells. There were no significantly different changes between the TB permeability of endothelium and smooth muscle cells exposed to either arachidonic or linoleic acid. Arachidonic acid leads to a 5-fold while linoleic acid leads to a 4-fold increase in cellular permeability to TB. On the other hand, palmitic acid (saturated fatty acid) caused little change but TB permeability of the smooth muscle appeared greater than that of the endothelial cells. Each of these changes could be reversed when the cells were washed after their incubation with free fatty acids but prior to the trypan blue albumin exposure. However, sustained changes in the endothelial permeability to TB were seen in the cells incubated with both arachidonate and H₂O₂. These

findings suggest that free arachidonate per se can transiently alter the permeability of cerebromicrovascular endothelium and/or serve as a substrate for peroxidation which leads to a persistent capillary endothelial injury. Both the release of arachidonate from the cellular membranes with or without peroxidation may alter the BBB function and in this way contribute to the development of cerebral edema during various disease processes occurring in the central nervous system.

b) Little information has been available about cerebromicrovascular carbohydrate metabolism and its responses to vasoactive substances which may alter the vascular reactivity. Our recent studies suggested that the norepinephrine (NE) induced glycogenolysis and the 5-hydroxytryptamine (5-HT) stimulated glycogenesis in separately cultured cerebromicrovascular endothelium and smooth muscle have distinct control mechanisms in each cell type. Lately we have demonstrated that glycogenolysis induced by addition of NE was partially prevented by butoxamine (β_2 -adrenergic blocker) in the endothelial cells but not in the smooth muscle cultures. The NE glycogenolysis in the smooth muscle, in contrast to that of the endothelium, appeared to be almost completely inhibited by exposure to ibuprofen (inhibitor of cyclooxygenase) and butoxamine when added to the medium prior to NE. These findings support the contention of neurogenic (adrenergic) regulation of the cerebral microvascular function which can be modulated by endogenous prostacyclin in the smooth muscle cells.

c) The collaborative studies with Drs. McCarron and McFarlin have recently shown that freshly isolated cerebromicrovascular endothelial cells (EC) obtained from SJL mice with EAE in contrast to EC separated from normal brain are I-A⁺ and can function as antigen presenting cells. However, the expression of Ia on the surface of normal EC can be induced by γ -interferon, (IFN γ) and subsequently the EC are capable of presenting antigen.

The current study developed and established EC cultures derived from microvessels of SJL mice susceptible to EAE. The dissociated endothelium was obtained from cerebral microvessels by homogenization, centrifugation, discontinuous sucrose gradient (1.0-1.5 M) and exposure to dispase (0.3%). The growth and propagation of the EC depended on fibronectin presence on the surface of plastic flasks and on endothelial cell growth supplement of the medium (GIBCO 199) containing fetal calf serum (20%). Samples from each generation of cells (1-6) stained positively with antibody to factor VIII antigen and failed to stain with anti-glial fibrillary acidic protein, antimacrophage and anti-IA. However the expression of I-A⁺ antigen and proliferative response of these cells was induced by exposure of the cells to IFN γ .

The expression of I-A molecules on cerebral vascular endothelium which normally do not express detectable levels of class II molecules may result in interaction with immune T cells and pathogenic creations which occur in certain brain diseases which are associated with T cells such as MS and EAE. Therefore, the establishment of murine (SJL) EC culture suitable for designing a model system to investigate the interaction between the cerebral EC and immune cells in living state, which are of importance in the understanding of the pathogenesis of several neurological disorders.

II. The continuous studies of cerebral ischemia, its pathophysiology, prevention and therapy have been focused on, a) the ischemic effect on the metabolic pathway of 5-HT and its relationship to the development and of brain edema, b) the relationship between energy charge, immediate source of energy and biogenic amines.

a) Our previous results demonstrated that the ischemic edema is associated with an increased release of 5-HT and kinetic changes in the properties of synaptosomal serotonin (S_2)-receptor binding sites. These changes were found at the time of greatest accumulation of tissue water and increased 5-HT release in the brain of gerbils subjected to bilateral carotid artery occlusion for 15 min and 1 hour release. To elucidate further the pathomechanism of cerebral ischemic edema and its association with changes in the 5-HT metabolic pathway we investigated the properties of 5-HT(1A+1B) and 5-HT(1B) receptor binding sites in the same model. Tritiated 5-HT in the presence of cold 5-HT served to detect 5-HT(1A+1B) binding sites while the addition of spiperone permitted the demonstration of 5-HT(1B) binding sites in synaptosomes of cerebral cortex. A significant reduction of 5-HT(1A+1B) and 5-HT(1B) binding sites was seen in the cerebrocortical synaptosomes obtained from brains of animals subjected to ischemia with and without reflow. In addition, the affinity of ^3H -5HT for 5-HT(1B) binding sites was increased as compared to controls. The kinetic changes in 5-HT(1B) binding sites suggest a presynaptic modulation of 5-HT autoreceptors to be present beside an altered function of post-synaptic 5-HT receptors [5-HT(1A+1B) and S_2 -receptors] in ischemic brain edema.

b) Changes in the brain content of biogenic amines belong to one of the main factors implicated in the pathogenesis of ischemic CNS damage. However, their role in pathomechanisms responsible for exacerbation of ischemic injury is still debatable especially since the relationship between the failure of energy and the disturbances in the monoamine pathway is unclear.

Until now, it has been rather difficult to correlate both events with certainty since the distribution of cerebral damage is not exactly reproducible in currently used cerebral stroke models. Therefore each

sample of the brain may include a variable amount of normal and partially ischemic tissue. The advantage of the present study was doublefold, because animals were killed by microwave irradiation that allowed the measurement of labile brain metabolites and biogenic amines from the same sample without ambiguity of post-mortem changes.

Both the energy charges and the content of ATP and P-creatine were reduced to the same extent in hippocampus and striatum at 5 min and 15 min of ischemia while the changes in monoamines were only found following deprivation of blood supply 15 min to the brain. On the other hand, in reflow the energy charge and the immediate source of energy recovered almost completely in these structures, whereas the changes in the content of the monoamines did not only persist but the level of NE was also reduced in recirculation after 5 min ischemia.

These results clearly demonstrate that the changes in the tissue energy charge are not directly associated with the disturbances of cerebral amines in ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02275-11 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cerebromicrovascular Endothelial Cultures: Interaction Between Prostaglandin & Adenosine Receptors linked to Adenylate Cyclase*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M. Spatz, M.D. Section Chief LNNS, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 6	PROFESSIONAL 2	OTHER 4
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The interaction between adenosine and PGE₂ receptors linked to adenylate cyclase was investigated in cultured endothelial cells derived from dissociated cerebral microvessels. PGE₂ stimulated adenylate cyclase in dose dependent manner (1nM-100µM) while adenosine increased the activity of adenylate cyclase only at high concentration of the drug (0.01-0.1mM). Increasing concentration of adenosine with PGE₂ (10⁻⁶M) had a statistically significant effect on adenylate cyclase stimulation only at lower doses of adenosine (1-10nM). The same concentrations of adenosine (1-10nM) alone did not enhance significantly the formation of cAMP. Those results indicate that both vasoactive substances adenosine and PGE₂ have an effect on receptors linked to adenylate cyclase. *[Formerly: Prostaglandin Receptors in Cultured Glia and Cerebromicrovascular Smooth Muscle]		
11 - LNNS/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02324-10 LNNS			
PERIOD COVERED October 1, 1986 through September 30, 1987					
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Blood-brain barrier: In vitro model for the study of cerebrovascular endothelial permeability					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> P.I. A. Villacara, M.D. M. Spatz, M.D. </td> <td style="width: 33%; vertical-align: top;"> Visiting Fellow Section Chief </td> <td style="width: 33%; vertical-align: top;"> LNNS/NINCDS LNNS/NINCDS </td> </tr> </table>			P.I. A. Villacara, M.D. M. Spatz, M.D.	Visiting Fellow Section Chief	LNNS/NINCDS LNNS/NINCDS
P.I. A. Villacara, M.D. M. Spatz, M.D.	Visiting Fellow Section Chief	LNNS/NINCDS LNNS/NINCDS			
COOPERATING UNITS (if any)					
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences					
SECTION Section on Neurocytobiology					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892					
TOTAL MAN-YEARS 0.7	PROFESSIONAL 0.3	OTHER 0.4			
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) The release of free fatty acids from cellular membranes has been implicated in attenuating the blood-brain barrier (BBB) permeability under pathological conditions. Recently we adopted a new <u>in vitro</u> system consisting of cerebromicrovascular endothelium grown on dextran microcarriers to study their properties related to the function of BBB under controlled conditions (Kempski and Spatz in press 1987). This method utilizes the fact that cell covered beads prevent accumulation of dyes like trypan blue(TB) in the microcarriers. Any increase in cellular permeability to TB results in reduced level of TB in the supernatant since TB that passes through the cellular layer binds tightly to the dextran beads. The aim of this investigation has been to use this model for evaluating the effects of free fatty acids on the permeability of separately cultured endothelial and smooth muscle cells derived from cerebral microvessels (Spatz et al. Brain Res, 191:577-582, 1980, 280:387-391, 1983). Both cell types excluded TB equally. There were no significantly different changes between the TB permeability of endothelium and smooth muscle cells exposed to either arachidonic or linoleic acid. Arachidonic acid leads to a 5-fold while linoleic acid leads to a 4-fold increase in cellular permeability to TB. On the other hand, palmitic acid caused little change but TB permeability of the smooth muscle appeared greater than that of the endothelial cells. Each of these changes could be reversed when the cells were washed after their incubation with free fatty acids but prior to the trypan blue albumin exposure. These results support the contention that free fatty acids can alter the permeability of cerebromicrovascular cellular elements and thus the function of BBB.					
12 - LNNS/DIR					

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02357-09 LNNS									
PERIOD COVERED: October 1, 1986 through September 30, 1987											
TITLE OF PROJECT (60 characters or less Title must fit on one line between the borders.) Cerebral Ischemia and Monoamines*											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.</td> <td style="width: 33%;">M. Spatz, M.D.</td> <td style="width: 33%;">Section Chief</td> <td style="width: 33%;">LNNS/NINCDS</td> </tr> <tr> <td></td> <td>K. Kumami, M.D.</td> <td>Visiting Fellow</td> <td>LNNS/NINCDS</td> </tr> </table>			P.I.	M. Spatz, M.D.	Section Chief	LNNS/NINCDS		K. Kumami, M.D.	Visiting Fellow	LNNS/NINCDS	
P.I.	M. Spatz, M.D.	Section Chief	LNNS/NINCDS								
	K. Kumami, M.D.	Visiting Fellow	LNNS/NINCDS								
COOPERATING UNITS (if any) B.B. Mrsulja, Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia											
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences											
SECTION Section on Neurocytobiology											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS .8	PROFESSIONAL: .1	OTHER .7									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) Human subjects</td> <td>☐ (b) Human tissues</td> <td>☒ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The relationship between the failure of energy and the disturbances in monoamine metabolites was investigated in the brain of Mongolian gerbils subjected to bilateral ischemia only (5 or 15 min) or with 1 hour reflow. The use of microwave irradiation to fix the brain allowed the measurement of labile metabolites and neurotransmitters from the same sample of tissue. Both the energy charge and the content of ATP and P-creatine were reduced to the same extent in the hippocampus and striatum at 5 min and 15 min of blood supply-deprivation to the brain while the changes in monoamines were only found after 15 min of ischemia. On the other hand, in reflow the energy charge and the immediate source of energy recovered almost completely in these structures whereas the changes in the content of the monoamines were not only present but the level of NE was also reduced in recirculation after 5 min ischemia. These results clearly demonstrate that the changes in the tissue energy charge are not directly associated with the disturbances of cerebral amines in ischemia. *[Formerly: "Effects of Ischemia on Synaptosomal Serotonin Uptake and Release"]											
14 - LNNS/DIR											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02361-10 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Investigations on Blood-Brain Barrier (BBB) Permeability		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. M. Spatz, M.D. Section Chief LNNS, NINCDS		
COOPERATING UNITS (if any) Prof. K.G. Go and Dr. H.J. Hauthoff, Department of Neurosurgery and Pathology, University of Groningen, The Netherlands		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0	PROFESSIONAL 0	OTHER 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Publication:</u> Go KG, Hauthoff HJ, Huitema S, Spatz M. Protein tracer permeability of the blood-brain barrier after transient cerebral ischemia in gerbils. Recent Progress in the Study and Therapy of Brain Edema, (eds.) KG Go and A Baethmann. Plenum Press, 1984:539-559.		
*This Project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02429-08 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Coordinate Changes in Brain Energy Metabolism and Protein Synthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) P.I. Thaddeus S. Nowak, Jr. Ph.D. Special Expert LNNS, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.1	0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This long term project investigating the integral relationship between <u>protein synthesis</u> and other aspects of <u>brain metabolism</u> has provided the <u>initial studies</u> on which two new projects are based, dealing with <u>stress protein induction</u> and <u>depletion of hippocampal dynorphin</u> after <u>ischemia</u>. These are outlined in detail elsewhere in this report. The role of <u>hyperthermia</u> in the reduction of <u>protein synthesis</u> after <u>amphetamine</u> administration has been demonstrated in mice, and correlated with enhanced drug-induced <u>brain glycogenolysis</u> at higher body temperatures. Current research continues to focus on ischemia, with a more direct emphasis on the regulation of protein synthesis as such. Effects of repeated ischemic intervals on brain protein synthesis are being investigated using the gerbil model currently available in the laboratory. Other studies will evaluate the role of initiation factor phosphorylation in the characteristic lasting reduction of protein synthesis after ischemia. On a more applied level it is considered that protein synthesis activity may be used as an index of ischemic stress in the preparation of tissue slices, providing a means for optimizing brain slices which may be applicable in a variety of physiological studies.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02463-07 LNNS

PERIOD COVERED

October 1, 1985 through September 30, 1987*

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Effect of Central Nervous Tissue on Cerebral Endothelial Properties

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.

M. Spatz, M.D.

Section Chief

LNNS, NINCDS

COOPERATING UNITS (if any)

Dr. Ronald F. Dodson, Division of Experimental Pathology, E. Tyler Chest Hospital, Tyler, Texas

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

*This Project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02548-06 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Evaluation of Electrical Impedance in Cerebral Ischemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I. H.G. Wagner, M.D. M. Seida, M.D. S. Xu, M.D. I. Klatzo, M.D.	Section Chief Visiting Fellow Visiting Fellow Chief	LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.2	PROFESSIONAL: 0.9	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) The effect of duration of ischemia on impedance in the caudate and cortex have been extensively studied in the cat and correlated with cerebral blood flow, edema, as well as other physiological parameters. It was found that when the cerebral blood flow was reduced to values below about 12 ml/100gm/min the cerebral impedance increased quickly within about 3 minutes to 150% or more of the pre-ischemic value. The magnitude of this rise was variable but the course was characteristic. After about 10 minutes the impedance rise leveled off to a plateau value. Upon release of the occlusion, the impedance fell within a few seconds towards pre-ischemic values. For short durations of ischemia (less than 10 minutes) the impedance fell to pre-ischemic values. For somewhat longer durations (about 20 minutes) the post-ischemic impedances values were significantly less than the pre-ischemic values. Confirmation that this lowered impedance was indicative of edema was found in lowered specific gravity of the brain tissues. Longer ischemic durations (one hour, and up to three hour durations) showed much variability in the course of post-ischemic impedances. However, all were found to be severely edematous by gravimetric analysis and showing severe leakage of Evans Blue dye through the blood-brain barrier. In some instances, a secondary rise in impedance appeared several hours after release of the occlusion and were believed to reflect a compression of the brain by edematous swelling. The relationship of changes in impedance to ischemia is not yet clear. Further study is planned.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02552-06 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Investigation of Extraneuronal Catechol Synthesizing Enzymes in the CNS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. M. Spatz, M.D. Section Chief LNNS, NINCDS		
COOPERATING UNITS (if any) Dr. Ikuko Nagatsu, Fujita-Gakuen Univ. School of Med., Toyoake, Aiche, Japan Dr. Toshiharu Nagatsu, Tokyo Institutes of Technology, Yokohama, Japan		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.1	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our previous immunohistochemical and biochemical studies of cerebral microvessels and cerebrovascular endothelial cultures showed the presence of phenylethanolamine-N-methyltransferase (PNMT) activity in both tissues. Since these extraneuronal tissues contain a catecholamine synthesizing enzyme which is responsible for conversion of norepinephrine to epinephrine, we extended these studies to determine whether vascular PNMT is indeed capable of producing epinephrine from norepinephrine. For this purpose a direct assay of endothelial epinephrine formed from norepinephrine was determined by using high pressure liquid chromatography. These studies, which are still in progress, have shown that the cultured cerebrovascular endothelium (2nd-4th generation) derived from dissociated cerebral microvascular fractions (obtained from rats) are capable of converting norepinephrine to epinephrine. *This Project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02572-05 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Effect of Abolition of BBB Opening on Water Content of Ischemic Brain Tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name title, laboratory, and institute affiliation)		
P.I P. Ting, M.D. T. Kuroiwa, M.D. I. Klatzo, M.D.	Special Expert Visiting Fellow Chief	LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.1	PROFESSIONAL 0.6	OTHER 0.5
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The effect of prevention of reactive hyperemia, which invariably follows release of arterial occlusion in areas of the brain subjected to ischemia of intensity below threshold level, was evaluated with regard to opening of the blood-brain barrier (BBB) associated with extravasation of serum proteins, and to development of ischemic brain edema. The reactive hyperemia was abolished by hypovolemic withdrawal of the blood at the time of recirculation. Such animals showed no opening of the BBB to proteins significantly reduced edema, when tested at 3 hours following recirculation, in comparison to edema in normovolemic animals subjected to similar intensity of one hour ischemia. Brain injury determined at 3 days after recirculation varied from none to moderate in cats with severe ischemia (below 12 mg/100g/min) in which reactive hyperemia and opening of the BBB was prevented by hypovolemia, whereas the cats with similar in severity ischemia, accompanied by reactive hyperemia and extravasation of EB, revealed a frank cerebral infarction. These studies demonstrate further the significance of serum protein extravasation in the development of brain edema and with regard to severity of ischemic injury. *This Project has been terminated.		
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02574-05 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987 *		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) A New Histochemical Method for the Detection of Adenylate Cyclase with Forskolin		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name title, laboratory, and institute affiliation) P.I. G. Szumanska, M.D. M. Spatz, M.D. Guest Worker Section Chief LNNS, NINCDS LNNS, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.3	PROFESSIONAL 0.4	OTHER 0.9
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided) A new histochemical method was developed for the detection of adenylate cyclase (AC) by stimulation of the enzyme activity with forskolin. This method was compared with the technique in which isoproterenol and 5-guanylylimidodiphosphate (GppNp) were used as activators of AC. The studies revealed that forskolin is not only a suitable activator of AC but is more effective than isoproterenol and GppNp for the demonstration of this enzyme histochemically. The availability of the method for the detection of AC activity without the necessity of using a hormonal stimulator has a great potential for the evaluation of this enzyme in normal and pathological tissues especially in those cases showing an absence or desensitization of the specific hormonal receptor linkage to AC. *This Project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02576-05 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cerebrovascular Smooth Muscle Cultures: Binding Studies of α_2 -Adrenergic Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	B. Wroblewska, Ph.D. M. Spatz, M.D.	Visiting Fellow Section Chief LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	0.8	1.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
The presence of α_2-adrenergic receptors was investigated in smooth muscle cell cultures using ^3H clonidine (α_2-adrenergic agonist) as a ligand. Specific binding sites of ^3H clonidine were defined as the excess over "blank" taken in the presence of $1\mu\text{M}$ "cold" clonidine. For the competitive studies different concentration of various adrenergic agonists and antagonists were used to displace binding with 4nM ^3H-clonidine. The rank order of potency for α-adrenergic agonists and antagonists was clonidine > phentolamine = yohimbine >> prazosin. The IC_{500} for the investigated displacers were: 25nM, 300nM $1\mu\text{M}$ and 9mM respectively. Competition curve produced by competing "cold" for ^3H-clonidine (4nM) showed a biphasic pattern indicative of multiple binding sites. Besides, the Scatchard analysis of saturation curve (concentration of ^3H-clonidine ranged from $6\mu\text{M}$ to .3 nM) and dissociation rate were characteristic of multiple population of the α_2-adrenergic binding sites in cultured smooth muscle cells. Thus, the existence of α_2-type adrenergic receptors not linked to AC activity observed in the cerebrovascular smooth muscle cells strongly suggest that their reactivity which is mediated by these receptors might be associated with Ca^{++} fluxes. *This Project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02620-04 LNNS												
PERIOD COVERED October 1, 1986 through September 30, 1987*														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Reactivity of Young Gerbil Brain to Cerebral Ischemia														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.</td> <td style="width: 33%;">H. Martinez, M.D.</td> <td style="width: 33%;">Visiting Associate</td> <td style="width: 33%;">LNNS, NINCDS</td> </tr> <tr> <td></td> <td>R. Cahn, M.D.</td> <td>Visiting Fellow</td> <td>LNNS, NINCDS</td> </tr> <tr> <td></td> <td>I. Klatzo, M.D.</td> <td>Chief</td> <td>LNNS, NINCDS</td> </tr> </table>			P.I.	H. Martinez, M.D.	Visiting Associate	LNNS, NINCDS		R. Cahn, M.D.	Visiting Fellow	LNNS, NINCDS		I. Klatzo, M.D.	Chief	LNNS, NINCDS
P.I.	H. Martinez, M.D.	Visiting Associate	LNNS, NINCDS											
	R. Cahn, M.D.	Visiting Fellow	LNNS, NINCDS											
	I. Klatzo, M.D.	Chief	LNNS, NINCDS											
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences														
SECTION Section on Cerebrovascular Pathology														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS 2.5	PROFESSIONAL 2.0	OTHER 0.5												
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Effects of 5 minute cerebral ischemia due to bilateral occlusion of the common carotid arteries were studies in 3 week old and adult gerbils. The biochemical assays revealed a considerable difference concerning the rate of depletion of the main energy metabolites indicating a slower energy metabolism in the young gerbils. Morphological studies carried out after 2 weeks revealed no evident ischemic injury in the young animals, whereas the brain of adult gerbils showed characteristic severe destruction of the CA1 sector of the hippocampus. The evaluation of regional cerebral blood flow (rCBF) with ¹⁴C iodoantipyrine radioautography revealed severe, uniform (below 10 ml/100g/min) ischemia in most of the both hemispheres, similar in intensity in both young and adult gerbils. The quantitative rCBF measurements based on hydrogen clearance and using implanted platinum electrodes indicated a marked hypoperfusion demonstrable at 30' and 1 hr recirculation periods in adult animals, whereas this was not present in young gerbils. Oxygen availability determinations indicated at 30 and 60 minutes recirculation a tissue hypoxia, much more pronounced in the adult than in the young animals. The studies indicate that among factors accounting for better tolerance of ischemia by young gerbils, in addition to slower energy utilization during initial stages of ischemia, considerably less pronounced tissue hypoxia during post-ischemic periods may play a significant role in pathomechanisms of ischemic injury. *This Project has been terminated.														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02623-04 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Ischemic Brain Edema: 5-HT Receptors*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: K. Kumami, M.D. M. Spatz, M.D. Visiting Fellow Section Chief LNNS, NINCDS LNNS, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 6	PROFESSIONAL: 4	OTHER: 2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Attenuation of kinetic properties of 5-HT (serotonin) S₂-receptor (³H-Ketanserin) binding sites was detected in tissue homogenate and synaptosomes of cerebral cortex in ischemic edema (Wroblewska et al 1985, 1986). The changes were found at the time of greatest accumulation of tissue water and increased 5-HT release in the brain of gerbils subjected to bilateral carotid artery occlusion for 15 min and 1 hour release. To elucidate further the pathomechanism of cerebral ischemic edema and its association with changes in the 5-HT metabolic pathway we investigated the properties of 5-HT(1A+1B) and 5-HT(1B) receptor binding sites in the same model. Tritiated 5-HT in the presence of cold 5-HT served to detect 5-HT(1A+1B) binding sites while the addition of spiperone permitted the demonstration of 5-HT(1B) binding sites in synaptosomes of cerebral cortex. A significant reduction of 5-HT(1A+1B) and 5-HT(1B) binding sites was seen in the cerebrocortical synaptosomes obtained from brains subjected to ischemia with and without reflow. In addition, the affinity of ³H-5HT for 5-HT(1B) binding sites was increased as compared to controls. The kinetic changes in 5-HT(1B) binding sites suggest a presynaptic modulation of 5-HT autoreceptors to be present beside an altered function of post-synaptic 5-HT receptors [5-HT(1A+1B) and S₂-receptors] in ischemic brain edema. *[Formerly "Ischemic Brain Edema: Effect of γ-hydroxybutyrate on S₂-Receptor Binding Sites]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02625-04 LNNS																								
PERIOD COVERED October 1, 1986 through September 30, 1987*																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Efficacy of PGBx to Protect Against Cerebral Ischemia																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.</td> <td style="width: 33%;">H.G. Wagner, M.D.</td> <td style="width: 33%;">Section Chief</td> <td style="width: 33%;">LNNS, NINCDS</td> </tr> <tr> <td></td> <td>B.B. Mrsulja, M.D.</td> <td>Visiting Scientist</td> <td>LNC, NINCDS</td> </tr> <tr> <td></td> <td>H. Martinez, M.D.</td> <td>Visiting Associate</td> <td>LNNS, NINCDS</td> </tr> <tr> <td></td> <td>H. Masaoka, M.D.</td> <td>Visiting Fellow</td> <td>LNNS, NINCDS</td> </tr> <tr> <td></td> <td>J. Dambrosia</td> <td>Biostatistician</td> <td>BFSB, NINCDS</td> </tr> <tr> <td></td> <td>I. Klatzo, M.D.</td> <td>Chief</td> <td>LNNS, NINCDS</td> </tr> </table>			P.I.	H.G. Wagner, M.D.	Section Chief	LNNS, NINCDS		B.B. Mrsulja, M.D.	Visiting Scientist	LNC, NINCDS		H. Martinez, M.D.	Visiting Associate	LNNS, NINCDS		H. Masaoka, M.D.	Visiting Fellow	LNNS, NINCDS		J. Dambrosia	Biostatistician	BFSB, NINCDS		I. Klatzo, M.D.	Chief	LNNS, NINCDS
P.I.	H.G. Wagner, M.D.	Section Chief	LNNS, NINCDS																							
	B.B. Mrsulja, M.D.	Visiting Scientist	LNC, NINCDS																							
	H. Martinez, M.D.	Visiting Associate	LNNS, NINCDS																							
	H. Masaoka, M.D.	Visiting Fellow	LNNS, NINCDS																							
	J. Dambrosia	Biostatistician	BFSB, NINCDS																							
	I. Klatzo, M.D.	Chief	LNNS, NINCDS																							
COOPERATING UNITS (if any) Hahnemann University, Philadelphia, PA																										
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences																										
SECTION Section on Cerebrovascular Pathophysiology																										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																										
TOTAL MAN-YEARS 0.5	PROFESSIONAL 0.4	OTHER 0.1																								
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In collaboration with Professor Thomas Devlin of Hahnemann University in Philadelphia, PA an effort was made to evaluate <u>a prostaglandin derivative called PGBx for its protective action against ischemic brain damage</u>. This compound, isolated in the course of studies on stress, was found by earlier workers to protect <i>vitro</i> mitochondrial oxidative phosphorylation from hypoxia. We compared the survival of PGBx treated and untreated adult mongolian gerbils which had been subjected to 15 minutes of bilateral carotid occlusion. Although our first set of studies showed beneficial effects, repeated studies with new supplies of PGBx failed to show the effect even with major variations in dosage and time of dosage. Although this conclusion does not invalidate the metabolic studies which had shown other beneficial effects such as the restoration of oxidative phosphorylation in mitochondria exposed to hypoxia, further studies have been deferred. *This Project has been terminated.																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02639-03 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Carbohydrate Metabolism in Cerebromicrovascular Cultures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Spatz, M.D. H. Yamamoto	Section Chief Guest Researcher LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any) David Lust, Case Western Reserve University, Cleveland, Ohio; B. B. Mrsulja, CVD Research Group, Institute of Biochemistry, Belgrade, Yugoslavia		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 8	PROFESSIONAL 5	OTHER 3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The regulation of norepinephrine induced glycogenolysis has been investigated in cultured cerebromicrovascular cellular elements. Glycogenolytic effect of norepinephrine was partially prevented by β_2-adrenergic blockers in endothelial cultures. On the other hand, the NE induced glycogenolysis was almost completely inhibited by exposure to ibuprofen and butoxamine (inhibitors of cyclooxygenase and β_2-adrenergic system). These findings support the contention of neurogenic (adrenergic) regulation of the cerebral microvascular function which can be modulated by endogenous prostacyclin of the smooth muscle cells.		
27 - LNNS/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02690-03 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Forskolin Effects on Glial and Cerebromicrovascular Cultures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P.I. M. Spatz, M.D. O. Kempfski, M.D. B. Wroblewska, Ph.D.	Section Chief Visiting Fellow Visiting Fellow	LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.3	PROFESSIONAL: 0.8	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) The role of cyclic nucleotides in growth control and differentiation is yet unresolved. The recent availability of forskolin, a drug which directly stimulates the catalytic subunit of adenylate cyclase, allowed to study growth regulation of brain cells by cAMP from a new viewpoint. Glial cells, endothelium and smooth muscle cells from brain microvessels were used for this <u>in-vitro</u> study. cAMP production, thymidine incorporation, and morphological and cytoskeletal changes were examined. The results demonstrated that in all three cell types, thymidine incorporation was reduced dose-dependently with maximal growth inhibition at 100µM forskolin. A one hour preincubation with forskolin abolished thymidine incorporation in fetal serum (FCS)-containing medium over the following 24 hours. Exposure to forskolin led also to drastic and immediate changes of cell morphology and F-actin composition in endothelium and smooth muscle. These changes were reversible. In glial cells morphologic changes were visible only after exposure to forskolin for more than 24 hours. These changes were accompanied by increased staining with antibodies against glial fibrillary acidic protein. The findings support a role of cAMP in growth regulation of these cells and indicate that forskolin might be used as a tool to induce growth arrest and eventually differentiation in cell cultures from mammalian brain. Publication: Kempfski O, Wroblewska B, Spatz M. Effects of forskolin on growth and morphology of cultured glial and cerebrovascular endothelial and smooth muscle cells. Int J Dev Neurosci. (in press) *Project has been terminated		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02691-03 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Ultrastructural observation on the Transvascular Route of Protein Removal in Vasogenic Brain Edema		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P.I.	I. Klatzo, M.D. R. Suzuki, M.D. T. Yamaguchi, M.D. H. Masaoka, M.D.	Chief Visiting Fellow Visiting Fellow Visiting Fellow LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any) Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. A.W. Vorbrott, M.D., Research Fellow H.M. Wisniewski, M.D., Director A.S. Lossinsky, M.D., Research Fellow		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.3	PROFESSIONAL 0.8	OTHER 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Our observations suggest that the observed, reverse, vesicular transport of HRP across the endothelial cells of some blood vessels represents one of several possible mechanisms responsible for the removal of extravasated proteins and of edematous fluid from brain extracellular space. This reverse transport is accompanied by a disruption of the surface anionic layer and changed polarity of endothelial cells manifested by the relocation of alkaline phosphatase activity from luminal to abluminal plasmalemma. The newly described mechanism for transvascular route of serum protein removal may play a very significant role in various phases of resolution of vasogenic edema and therefore investigations concerning possible acceleration of such transvascular removal may be of importance in designing some therapeutic measures in vasogenic brain edema.		
*This Project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02693-03 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987*		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Features of the Blood-Brain Barrier(BBB) Opening to Horseradish Peroxidase(HRP) at the Onset of Bicuculline		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I. H. Laursen, M.D. G. Goping I. Klatzo, M.D.	C. Nitsch, M.D. Visiting Associate Lab. Technician Chief	LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.3	PROFESSIONAL: 0.8	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) Rabbits which had received horseradish peroxidase (HRP) intravenously were subjected to bicuculline-induced generalized seizures of 15 min duration in order to elucidate the mechanism by which macromolecules traverse the blood-brain barrier (BBB) in specific neuroanatomically defined brain areas. As a rule, transendothelial pinocytosis at the level of arterioles was the main route of passage. In addition, tracer-filled pinocytotic vesicles were also observed in the capillaries of the hippocampus, thalamus, whereas in thalamus, hypothalamus and septum, they were present also in the endothelium of venules. Indication of an opening of the interendothelial tight junctions was found in the hypothalamus. The HRP, which had reached the neuropil due to the seizure-evoked BBB opening, accumulated in the interstitial spaces and penetrated the synaptic cleft region. Uptake of the tracer in vesicular form into presynaptic boutons, presumably excitatory ones, was observed in all brain regions; it was frequent in pallidum, hippocampus and medulla oblongata and very intense in the thalamus. Concomittant uptake in postsynaptic dendrites, was present mostly in the vicinity of boutons. Incorporation into glial processes was rare. It is concluded, that blood-borne macromolecules traverse the BBB by regionally selective, transmitter-controlled pinocytotic transport and that the neuronal uptake of the foreign protein during the generalized seizures is at least partially dependent on the involvement of synapses of particular brain regions. These studies contribute to the understanding of mechanisms operative in epileptic seizures. *This Project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02694-02 LNNS
PERIOD COVERED <u>October 1, 1986 through September 30, 1987*</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) <u>Catecholamine Degradating Enzymes in Cerebromicrovascular Cultures</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. M. Spatz, M.D. Section Chief LNNS/NINCDS		
COOPERATING UNITS (if any) C.R. Creveling, Laboratory of Bioorganic Chemistry, NIAMDD; I. Nagatsu, Fujita-Gaknen University School of Medicine, Toyoka, Japan; T. Nagatsu, Nagoya School of Medicine, Nagoya, Japan		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.0	PROFESSIONAL: .8	OTHER: .2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) The activity of catechol-O-methyltransferase (COMT) was investigated in cultured and propagated cerebromicrovascular endothelial and smooth muscle cells using high performance liquid chromatography and immunocytochemistry. The existence of COMT was detected in both cell types. The demonstration of this enzyme activity in the cerebromicrovascular smooth muscle cells, in addition to the endothelium, indicates that the enzymatic barrier to catecholamine is not limited to capillaries, the main constituents of the blood-brain barrier. <u>Publication:</u> Spatz M, Kanedo N, Sumi Ch, Nagatsu I, Creveling CR, Nagatsu T. The presence of catechol-O-methyltransferase activity in separately cultured cerebromicrovascular endothelial and smooth muscle cells. Brain Res 1986;381:363-367. *This project has been temporarily suspended due to loss of cell cultures (defective incubator).		
31 - LNNS/DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02704-02 LNNS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Transient Ischemic Attacks (TIAs) in the Experiment Gerbil Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	S. Tomida M.D.	Visiting Fellow	LNNS, NINCDS
	T.S. Nowak, Jr. Ph.D.	Special Expert	LNNS, NINCDS
	K. Vass, M.D.	Visiting Associate	LNNS, NINCDS
	J. Lohr	Biological Lab. Technician	LNNS, NINCDS
	I. Klatzo, M.D.	Chief	LNNS, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.4

PROFESSIONAL:

1.0

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The experimental model for TIAs, developed in gerbils, represents the first model which can be used in an easy, reproducible way to study systematically various aspects concerning the effect of repeated ischemic attacks upon brain tissue. Our observations using this model indicated that there is a cumulative effect of repeated ischemic insults, when they are carried out at time intervals at which there is post-ischemic hypoperfusion. The cumulative effect of repeated ischemic insults is expressed in intensity of edema and brain tissue injury, which in 3 repeated insults is considerably higher than resulting from a single ischemic insult of duration equal to sum of individual repetitive attacks.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02718-02 LNNS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (60 characters or less: Title must fit on one line between the borders)

Study of Cerebral Electrical Activity Associated with Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.

H.G. Wagner, M.D.

Section Chief

LNNS/NINCDS

S. Xu, M.D.

Visiting Fellow

LNNS/NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathophysiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A study of the electrophysiological manifestations of ischemic damage to functional activity. It is expected that the initial focus of effort will be on the recovery of electrical activity following temporary ischemic insults of graded durations. Since initiation of this project, instrumentation has been procured and installed but collection of experimental data has not commenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02720-01 LNNS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Stress Protein Induction in Gerbil Brain after Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name title, laboratory, and institute affiliation)

PI:	Thaddeus S. Nowak, Jr., Ph.D.	Special Expert	LNNS, NINCDS
	Karl Vass, M.D.	Visiting Associate	LNNS, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| ☐ (a) Human subjects | ☐ (b) Human tissues | ☒ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since the initial demonstration of induction of the major mammalian stress (heat shock) protein, hsp70, after transient ischemia, efforts have been directed toward a) quantitating increased transcription of hsp70 and other stress-inducible mRNAs after ischemia, and b) immunocytochemical localization of hsp70 induction in gerbil brain. Induction of both hsp70 and ubiquitin mRNA sequences have now been demonstrated after ischemia using cDNA probes. Ubiquitin mRNA is only modestly induced (70% increase) and peaks at 6 hr recirculation. Hsp70 mRNA is more strongly induced (more than 2-fold) and peaks at 12 hr, coincident with the time of maximal hsp70 translation previously determined. A striking temporal and anatomical pattern of hsp70 induction was demonstrated in post-ischemic brain using monoclonal antibodies considered specific for inducible forms of the hsp70 family. Control animals showed immunoreactivity which was restricted to ependymal cells lining the ventricles, and this remained unchanged after ischemia. During 24 hr recirculation after 10 min ischemia, hsp70 immunoreactivity appeared in dentate granule cells, in neurons of habenula and striatum, and in a number of basal forebrain and cortical regions. At 48 hr intense reactivity was evident in CA3 neurons of hippocampus. By 96 hr immunoreactivity was restricted to some residual staining of CA3 with strong labeling of entorhinal and other cortical regions. It is of interest that positive cells are found in hippocampus and other limbic structures, circuitry which may be the anatomical substrate for proposed excitotoxic mechanisms of neuronal loss after ischemia. Accumulation of hsp70 was apparent in CA3 and dentate granule cells which survive transient ischemia, but was greatly attenuated in CA1 neurons which do not. This heterogeneity in hsp70 induction may reflect variations in post-ischemic metabolic stress as well as in the response to stress of various cell populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02721-01 LNNS
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Hippocampal Dynorphin Levels and Synthesis After Ischemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: T. S. Nowak, Jr., Ph.D. Special Expert LNNS, NINCDS		
COOPERATING UNITS (if any) Brian Cox, Uniformed Services University of the Health Sciences Jacqueline McGinty, East Carolina University School of Medicine		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.3	PROFESSIONAL: 0.1	OTHER 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Peptides</u> derived from each of the <u>endogenous opioid</u> precursors were measured in <u>gerbil brain regions</u> after <u>transient ischemia</u>. Lasting depletion of <u>dynorphin A immunoreactivity</u> were observed only in <u>hippocampus</u>. Levels of hippocampal dynorphin fell by 30-40% as early as 1 hr after recirculation and remained at 50% of control for at least one week. In some experiments peptide levels showed a transient recovery at 24 hr. Preliminary results suggest a similar reduction in hippocampal pro-dynorphin mRNA levels with a similar time course. These results demonstrate at the neurochemical level a unique sensitivity of the dynorphin-containing dentate granule cell / mossy fiber pathway to transient ischemia. Although these cells remain histologically intact, the decrease in dynorphin precedes and continues during the delayed loss of hippocampal CA1 neurons characteristic of this model and further defines the selective vulnerability of hippocampal circuitry following ischemia. The mechanisms responsible for peptide depletion, and the functional roles of dynorphin peptides in the physiology and pathology of the hippocampus, remain to be elucidated.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02722-01 LNNS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Comparison of Hydrogen Clearance and Tracer Diffusion Methods for Determining Cerebral Blood Flow

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.	Thaddeus S. Novak, Jr., Ph.D.	Special Expert	LNNS, NINCDS
	Shuichi Tomida, M.D.	Visiting Fellow	LNNS, NINCDS
	Junichi Ikeda, M.D.	Visiting Fellow	LNNS, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20392

TOTAL MAN-YEARS

0.6

PROFESSIONAL:

0.4

OTHER

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Recent results in the gerbil have demonstrated dramatic reductions of hemispheric blood flow, assessed by a [^3H] nicotine tracer diffusion method, following placement of a single electrode in striatum for the purpose of measuring blood flow by the alternative hydrogen clearance method. Blood flow in cortical regions of the impaired hemisphere is reduced several-fold relative to the opposite hemisphere, returning gradually to control levels by 3-6 hr. Blood flow measurements in striatum, obtained with the implanted hydrogen clearance electrode, also increases two-fold during 6 hr after placement. These results demonstrate that significant reductions of normal blood flow arise following acute placement of electrodes for hydrogen clearance measurements in the gerbil, although chronic implantation may still provide valid measurements.

Measurements obtained in cats several years ago with chronically implanted hydrogen electrodes gave unexpectedly high values for striatal blood flow, attributed at that time to the light anaesthesia which could be used at the time of measurement. These results will be systematically reinvestigated in view of the current findings in the gerbil.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02741-01 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Study of Oxygen Tension Change Associated with Ischemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) P.I. H.G. Wagner, M.D. S. Xu, M.D. Section Chief Visiting Fellow LNNS/NINCDS LNNS/NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.6	OTHER 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Using polarographic electrodes, oxygen tensions were measured in the cerebral cortex of gerbils subjected to repeated ischemias of short duration. In controls before ischemia, the cortex pO₂ values were found to vary from about 55 to zero tor. These variations did not seem to correlate with depth or topographical location. When this data was assembled into a bar histogram of frequency of particular O₂ tensions, the distribution was gaussian shaped with its mean in the 20-25 tor interval. When the animal was made ischemic by bilateral carotid artery occlusion, the pO₂ dropped rapidly to zero, reaching this value in about 3 seconds. The dynamics of the recovery of the pO₂ was dependent on the duration of ischemia. Very short duration ischemias of a few seconds were followed by immediate recovery of pO₂ in a few seconds. Longer durations of ischemia (up to one minute) the pO₂ level recovers promptly with some overshooting. The PO₂ level rises above the pre-ischemic level by as much as twofold. With still longer durations of ischemia, (1 minute to 5 minutes), the pO₂ level rises sluggishly and often a delay in any observable sign of pO₂ recovery. Overshoot of pO₂ values above the preischemic values are not seen.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02744-01 LNNS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Microvascular Disturbances and Edema Formation After Repetitive Ischemia of Gerbil Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	K. Vass, M.D.	Visiting Associate	LNNS, NINCDS
	S. Tomida, M.D.	Visiting Fellow	LNNS, NINCDS
	K.-A. Hossmann, M.D.	Guest Researcher	LNNS, NINCDS
	T.S. Nowak, Ph.D.	Special Expert	LNNS, NINCDS
	I. Klatzo, M.D.	Chief	LNNS, NINCDS

COOPERATING UNITS (if any)

Max-Planck Institute for Neurologic Research, Cologne, West Germany

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.1

PROFESSIONAL:

0.9

OTHER

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The nature of the cumulative effect of three repeated 5 minute occlusions of the common carotid artery in gerbil, carried out during the period of post-ischemic hypoperfusion was studied by visualization of cerebral microcirculation with intravenously injected Evans Blue(EB) tracer. The animals were sacrificed shortly after EB injection and the distribution of the tracer in the capillaries and larger cerebral blood vessels was studied in frozen sections under the fluorescence microscope. Repetitive ischemia caused progressively increasing brain edema and progressive reduction of the number of perfused capillaries. Immediately, after each ischemic episode transient recruitment of capillaries occurred, thus excluding no-reflow as a main pathogenic factor of microcirculatory disturbances. The pattern of microcirculation 6 and 24 hours after the last occlusion revealed a redistribution of circulating blood, characterized by a reduction in the number of EB-filled capillaries and, at the same time, noticeable dilatation of the larger vascular channels. The redistribution of microcirculatory flow was shown to be associated with the normal cerebral blood flow levels at that time. Our studies suggest a closed interrelationship between post-ischemic microcirculatory hypoperfusion and the development of brain edema, the degree and extent of which progresses with the repetition of ischemic episodes, when they are carried out during the periods of hypoperfusion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02745-01 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effect of Aminophylline on Post-Ischemic Edema and Brain Injury		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I. M. Seida, M.D. H.G. Wagner, M.D. I. Klatzo, M.D.	Visiting Fellow Section Chief Chief	LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.6	PROFESSIONAL 0.5	OTHER 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Effect of aminophylline on post-ischemic edema and brain injury was studied in cats subjected to the middle cerebral artery (MCA) occlusion for 1 hour. Administration of aminophylline 22.8 mg/kg infused for 15 minutes at the time of release of MCA occlusion resulted in suppression of reactive hyperemia and proved to have an unequivocal beneficial effect on development of post-ischemic edema and brain injury. The animals with aminophylline showed no breakdown of the blood-brain barrier to protein tracers and showed no significant edema in the ischemic regions, when sacrificed 3 hours after release of MCA occlusion. Also, cats with aminophylline treatment revealed significantly milder ischemic injury when studied histologically at 3 and 14 day periods after recirculation. Our studies thus demonstrate a marked beneficial effect of aminophylline with a potential to be considered for treatment of patients in certain clinical conditions.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02749-01 LNNS									
PERIOD COVERED October 1, 1986 through September 30, 1987											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Measurement of Cerebral Blood Flow by Laser Doppler Velocimetry											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">P. I. H.G. Wagner, M.D.</td> <td style="width: 40%;">Section Chief</td> <td style="width: 20%;">LNNS/NINCDS</td> </tr> <tr> <td>S. Xu, M.D.</td> <td>Visiting Fellow</td> <td>LNNS/NINCDS</td> </tr> <tr> <td>R.F. Bonner</td> <td>Biomedical Engineer</td> <td>BEIB, RS, NIH</td> </tr> </table>			P. I. H.G. Wagner, M.D.	Section Chief	LNNS/NINCDS	S. Xu, M.D.	Visiting Fellow	LNNS/NINCDS	R.F. Bonner	Biomedical Engineer	BEIB, RS, NIH
P. I. H.G. Wagner, M.D.	Section Chief	LNNS/NINCDS									
S. Xu, M.D.	Visiting Fellow	LNNS/NINCDS									
R.F. Bonner	Biomedical Engineer	BEIB, RS, NIH									
COOPERATING UNITS (if any) Biomedical Engineering, Research Services, NIH											
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences											
SECTION Section on Cerebrovascular Pathophysiology											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1									
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) A study was made of cerebral blood flow (CBF) in gerbils by Laser doppler velocimetry (LDV). Each animal was subjected to five minute bilateral carotid occlusions each hour for 3 hours. Continuous measurement of CBF was made of the parietal cortex through the cranium. There was excellent consistency and repeatability from gerbil to gerbil. The essential features were: The CBF as revealed by LDV, dropped to zero during ischemia, arose upon release of ischemia to a value slightly above the pre-ischemic value, then declined to a much lower value over the hour before the next occlusion. There was no evidence of an accumulative effect with respect to the ischemia.											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02750-01 LNNS
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Observations on Cerebral Ischemia at Injury Threshold Levels		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P.I. M. Seida, M.D. S. Tomida, M.D. H.G. Wagner, M.D. I. Klatzo, M.D.	Visiting Fellow Visiting Fellow Section Chief Chief	LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.6	OTHER 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.) Studies on mechanisms, associated with survival of the neurons subjected to ischemia, have important clinical implications, since elucidation of the nature of such mechanisms may ameliorate or prevent brain tissue damage following cerebral ischemic insults. In experimental research, the degree of ischemic injury which allows recovery of affected neurons is called "penumbra", i.e. resembling a twilight zone around solar eclipse. In our studies, we produced conditions of penumbra by subjecting cats to 20 minute occlusion of the middle cerebral artery (MCA) and studying various parameters of ischemic injury up to 2 weeks after the insult. Our studies demonstrated that although the animals showed significant and widespread changes up to 3 days after recirculation, there was either complete or partial recovery with regard to edema and neuronal damage, when animals were examined 2 weeks after MCA occlusion. Our investigations resulted thus in establishing an experimental model where pathomechanisms associated with reversible ischemic damage could be analyzed and, potentially, manipulated.		
41 - LNNS/DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02751-01 LNNS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Interaction between Cerebral Capillary Endothelium and Immune Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.

M. Spatz, M.D.

Section Chief

LNNS, NINCDS

COOPERATING UNITS (if any)

R.M. McCarron and D.E. McFarlin, Neuroimmunology Branch, NINCDS

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Recently, we have shown that freshly isolated cerebromicrovascular endothelial cells(EC) obtained from SJL mice with EAE in contrast to EC separated from normal brain are I-A⁺ and can function as antigen presenting cells. However, the expression of Ia on the surface of normal EC can be induced by γ -interferon, (IFN γ) and subsequently the EC are capable of presenting antigen (J Immunol 1985; 134:3100 and 1986; 137:3428). This study centered in developing and establishing EC cultures derived from microvessels of SJL mice susceptible to EAE. The dissociated endothelium was obtained from cerebral microvessels by homogenization, centrifugation, discontinuous sucrose gradient (1.0-1.5 M) and exposure to dispase (0.3%). The growth and propagation of the EC depended on fibronectin presence on the surface of plastic flasks and on endothelial cell growth supplement of the medium (GIBCO 199) containing fetal calf serum (20%). Samples from each generation of cells (1-3) stained positively with antibody to factor VIII antigen and failed to stain with anti-glial fibrillary acidic protein, anti-macrophage and anti-IA. However the expression of IA⁺ antigen and proliferative response of these cells was induced by exposure of the cells to IFN γ . These findings indicate that the established murine EC culture is suitable for designing a model system to investigate the interaction between the cerebral EC and immune cells in living state, which are of importance in the understanding of the pathogenesis of several neurological disorders.

ANNUAL REPORT

October 1, 1986 through September 30, 1987
Laboratory of Neurophysiology

National Institute of Neurological and
Communicative Disorders and Stroke

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Cell Biological Studies of CNS Neurons, Pituitary and Immune Cells Z01 NS 02330-10 LNP	2
Synaptic Contacts of Retinal Neurons Z01 NS 01659-19 LNP	3
Structure and Function in Retinal Neurons Z01 NS 02631-04 LNP	4
Anatomical Studies of Neurons, Neurotransmitters and Neurotransmitter Receptors Z01 NS 02705-02 LNP	5

Laboratory of Neurophysiology, DIR
National Institute of Neurological and
Communicative Disorders and Stroke
Jeffery L. Barker, M.D., Chief

During FY 87 research carried out by members of the Laboratory of Neurophysiology continued along two complementary and necessary directions. One line of direction involved sustained application of established, though contemporary techniques in cellular physiology. The other line included development of new and innovative strategies for studying signal transduction mechanisms which is the focus, directly or indirectly, of most, if not all of the experimentation.

The goal of the research program is to understand specific signal transduction mechanisms in the larger context of intercellular communication, especially that involving transmitter substances. All cells studied to date exhibit some form of membrane excitability, which is a common site of many transduction mechanisms. The developmental expression of such mechanisms in nerve, endocrine and immune cells marks an important and critical period in phenotypic differentiation that is maintained throughout life. Thus, some of the current activity is based on material derived from the embryonic and early post-natal period of development, a largely unexplored area that includes quite significant and rapid changes in cell structure and function. By probing in a multi-disciplinary way for changes in the differentiation of specific signal transduction mechanisms during the development of nerve, endocrine and immune systems it should be possible to gain fundamental insights into trophic and transmitter functions as specific cell lineages become phenotypically committed. In this way it may be possible to describe the cellular distribution and functional roles of certain transduction mechanisms and begin to decipher certain aspects of the "transmitter code".

All of the projects are at cellular or molecular levels of study. Specific strategies include: 1) monolayers of primary nerve and glial cells cultured from embryonic mammalian central nervous system, from anterior pituitary and intermediate lobe and from adrenal medulla; 2) monolayers of clonal cells expressing pituitary and immune functions; 3) quantitative electrophysiological analysis of cellular excitability expressed in culture; 4) quantitative electrophysiological analysis of cellular excitability resident in the vertebrate retina and hippocampus; 5) flow cytometric analysis of physiologically important properties exhibited in populations of nerve, endocrine and immune cells; 6) flow cytometric isolation of specific nerve, endocrine and glial elements; 7) light- and electron-microscopic resolution of cellular form and subcellular structure in normally developed mammalian CNS tissues including retina and spinal cord and clonal pituitary cells; 8) immunohistochemical characterization of transmitter phenotype and surface receptor expressions in mammalian CNS tissue in vivo and in monolayer culture; and 9) image analysis processing of cultured cell morphology and physiology. Conceptually, these diverse cellular strategies harmonize to allow quantitative resolution of signal transduction mechanisms. The strength of the Laboratory's research enterprise then lies in large part in the spectrum of innovative strategies and setting for multi-disciplinary study.

Two developments during FY 87 have especially long-term implications. The first involves fluorescence microscopic identification of dopaminergic neurons differentiating in monolayer cultures of heterogeneous CNS cells by Drs. Mariani and Silva. This development will allow careful cellular study of a rare but functionally important central neuronal phenotype from embryogenesis to maturity. The second includes refinement and application of a voltage-sensitive indicator dye technology for probing the electrophysiological and optical properties of entire cell populations using a fluorescence-activated cell sorter (FACS) by Drs. Mandler, Schaffner and Lange and Ms. Novotny. The advent of "flow physiology" on the FACS provides a novel perspective for studying the development and cellular distribution of excitable membrane properties. With this strategy it is possible to identify the developmental schedule of specific membrane excitabilities, including receptor and ion channel functions. This strategy complements well-established single-cell electrophysiological recording methods routinely used in the Laboratory for elucidating excitable membrane properties expressed in acutely isolated or cultured cells. It may well provide an opportune avenue for discovering the role of specific membrane excitabilities in various CNS functions at an exceedingly expeditious rate.

The Laboratory of Neurophysiology has continued to develop new strategies to study the physiology of nerve cells. However, the strategies in development are applicable to problems involving most, if not all, cells since all cells exhibit membrane excitability and mechanisms elaborated to transduce input signals into output functions. Hence, these strategies are being utilized to study such mechanisms expressed in phenotypically distinct cell types. In time it should be possible to compare physiologically differentiated signal transduction mechanisms resident in specific cell types with pathologically altered ones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02019-15 LNP
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Electrophysiological Studies on Membrane Excitability		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) J.L. Barker, Chief, LNP, DIR, NINCDS. Others (LNP, DIR, NINCDS): T.G. Smith, Unit Head; G.D. Lange, Physiologist; M.K. Walton, Senior Staff Fellow; P.A. Sheehy, Staff Fellow; O.L. Lewis, Senior Staff Fellow; N.L. Silva, NRC Fellow; J. ffrench-Mullen, NRC Fellow; N.L. Harrison, Fogarty Fellow; S.V.P. Jones, Fogarty Fellow; R.W. Turner, Guest Researcher; David Bekelman, Guest Researcher		
COOPERATING UNITS (if any) D.E.R. Meyers (LNLC, NINCDS); W.B. Marks (LNLC, NINCDS); M. Zatz (LCB, NIMH); W.H. Sherriff (RSB, DIR, NIMH); B. Dufy (Laboratoire de Neurophysiologie, Universite de Bordeaux II, Bordeaux, France); B. Whitehead (Cornell University)		
LAB/BRANCH Laboratory of Neurophysiology, DIR, NINCDS		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 12	PROFESSIONAL 11	OTHER 1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The aim of this research program involves elucidation of the ion permeability mechanisms expressed by primary cells cultured from the embryonic mammalian CNS, from endocrine pituitary and from immune tissues. These mechanisms are considered critical in the physiology and diverse functions of the various cellular phenotypes. Specific lines of investigation include projects on embryonic CNS neurons cultured from spinal and supraspinal regions, clonal and primary pituitary cells and clonal and primary effector lymphocytes as well as their tumor targets. Electrophysiological measurements of excitability are made in membrane patches or in single cells using either low-resistance patch-clamp pipettes or high-resistance microelectrodes for recording. The different assay techniques provide complementary data for characterizing the membrane and cytoplasmic mechanisms underlying ion conductances in these cells. Principal observations this year include the following: 1) cultured hippocampal neurons utilize GABA to signal inhibitory outflows that exhibit a four-fold range in their time course; 2) some GABAergic neurons exhibit a recurrent form of synaptic inhibition that regulates their output; 3) synaptic transmission of GABA signals at GABA _A receptors coupled to Cl ⁻ channels is depressed by activation of GABA _B receptors at pre-synaptic terminals; 4) anesthetic steroids do not share barbiturate block of voltage-gated Ca ²⁺ conductance; 5) putative dopaminergic neurons cultured from the mesencephalon exhibit a distinctive set of excitable membrane properties, an after-depolarization being one prominent and consistent feature; 6) voltage-activated Ca ²⁺ currents differ in FACS-sorted prolactin and growth hormone cells; 7) clonal pituitary cell capacitance rises as cells hyperpolarize to secretagogue; 8) voltage-dependent Ca ²⁺ channels regulate melatonin secretion from chick pineal cells; 9) antigen crosslinking of receptors on clonal immune cells triggers a transient depolarization that can be mimicked by dialyzing cells with inositol trisphosphate and tetraakisphosphate.		
1 - LNP, DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02330-10 LNP
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cell Biological Studies of CNS Neurons, Pituitary and Immune Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.L. Barker, Chief, LNP, DIR, NINCDS. Others (LNP, DIR, NINCDS): U. DiPorzio, Visiting Scientist; G.D. Lange, Physiologist; R.N. Mandler, Visiting Scientist; E. A. Novotny, Biologist; P.A. St.John, Senior Staff Fellow; A.E. Schaffner, Senior Staff Fellow; R. Cruciani, Visiting Associate; M. Fiszman, Visiting Fellow; V. Smallwood, Bio. Lab. Technician		
COOPERATING UNITS (if any) M. Fordis (LMB, NCI); B. Howard (LMB, NCI); P. Kushner (ALS Fdn., San Francisco); T. Behar (LMG, NINCDS); M. Dubois-Dalcq (LMG, NINCOS); S. Schinelli (Sec. Immunopharm., NINCDS); A. Zuddas (Sect. Immunopharm., NINCDS)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 10	PROFESSIONAL: 6.5	OTHER: 3.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The immediate and continuing aim of this line of investigation involves the development of strategies to probe the development and differentiation of <u>phenotypic properties</u> expressed by <u>functionally distinct types</u> of nerve, endocrine and <u>immune cells</u>, especially those involved in signal transduction mechanisms. The strategies in development include <u>dual-laser fluorescence-activated cell analysis</u> and sorting (FACS), <u>light-microscopic characterization of cells in monolayer culture</u> using immunoreagents reacting with cytoplasmic and surface antigens and <u>quantitative analysis of optically detectable fluorescence signals</u> emanating from cells in monolayer culture. Principal observations this year include: 1) further refinement of a voltage-sensitive indicator dye protocol for assaying membrane potential distributions in heterogeneous suspensions of embryonic and early postnatal cells from the mammalian CNS; 2) discovery of the development of functional GABA receptors coupled to K⁺ conductance mechanisms before the appearance of GABA receptors coupled to Cl⁻ conductances; 3) discovery of the developmental time course of voltage-gated Na⁺ conductance, which occurs following the appearance of functional GABA receptors; 4) discovery of the expression of excitatory aminoacid receptor functions developing after the appearance of voltage-gated Na⁺ conductances; 5) <u>localization of the functional excitatory aminoacid receptors to the ventral region of the developing spinal cord</u>; 6) FACS analyses of <u>cell-cycle dynamics in co-transfected (CV-1) monkey kidney cells</u>; 7) computer-assisted analysis of <u>Ca²⁺ indicator dye signals</u> emanating from FACS-sorted growth hormone and prolactin cells; 8) biochemical and autoradiographic dissection of <u>MPTP dynamics in cultured astrocytes and catecholamine neurons</u>; 9) development of a quinacrine staining protocol for use with FACS and light microscopic analysis of <u>secretory activity</u>; 10) FACS isolation of <u>O-2A glial progenitors</u>.		

2 LNP, DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01659-19 LNP
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synaptic Contacts of Retinal Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: A. Lasansky, Head, Unit on Cell Biology, LNP, DIR, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology, DIR, NINCDS		
SECTION Unit on Cell Biology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Recordings in the whole-cell configuration from <u>salamander cones</u> in retinal slices lack the depolarizing component associated with <u>horizontal cell</u> feedback. Possible reasons for this observation are horizontal cell damage caused by the slicing and loss of essential cytoplasmic components through diffusion into the electrode. Whole-cell recordings from <u>bipolar cells</u> have continued to be infrequently achieved due to instability of the giga-ohm seals.		
3 LNP, DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02631-04 LNP
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Structure and Function in Retinal Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: Ralph R. Nelson Physiologist LNP, NINCDS Others: Andrew P. Mariani Special Expert LNP, NINCDS Michael Freed Guest Researcher LNP, NINCDS Renata Pflug Instructor Physiology Dept. U. of Vienna Jan N.M. de Melo Visiting Associate LNP, NINCDS E. K. Barbehenn Pharmacologist DMEDP, FDA Eberhart Zremer Professor Eye Clinic Lud.-Max.-U., Munich Steven Baer Staff Fellow MR, NIDDK		
COOPERATING UNITS (if any) Division of Metabolic and Endocrine Drug Products, FDA; (E.K. Barbehenn); Department of Physiology, University of Vienna, Austria (Renate Pflug); Ludwig-Maximilians- University, Eye Clinic Munich, FRG (Eberhart Zremer).		
LAB/BRANCH Laboratory of Neurophysiology, DIR, NINCDS		
SECTION Neural Circuitry Unit		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In the <u>photoreceptor</u> layer of <u>Rana pipiens</u>, <u>cGMP</u> levels have been measured <u>in vivo</u> using a rapid freezing technique. In darkness mean concentrations of 57 pmol kg⁻¹ were obtained, but after extended periods of illumination levels dropped to 9 pmol kg⁻¹. Brief (100 msec) flashes produced barely significant (10-15%) declines; minimum concentrations were reached only after several hours of dim illumination. The results suggest that the bulk of cGMP decline reflects a long term biochemical readjustment of <u>rods</u> to background illumination rather than a <u>phototransductive</u> message. In the <u>outer plexiform</u> layer of the cat retina background stimuli enhance the responses of <u>horizontal cells</u> to small <u>red flickering</u> spots. The responses to the spots originate with the <u>red cones</u>; the <u>enhancement</u> effect of the background originates with <u>rods</u>, as demonstrated by <u>spectral sensitivity</u> studies. The effect diminishes approximately exponentially with the width of either square or slit stimuli (space constant 250-400 um), but is larger with squares, both results consistent with <u>theoretical models</u> of voltage attenuation of background induced surround signals which decrement into the test region through the <u>horizontal cell syncytium</u>. The enhancement appears to result from the direct action of the <u>horizontal cells</u> on <u>cones</u>. There are also suggestions of similar horizontal cell mediated interactions among different cone types. The effects of <u>light</u> on the <u>input resistances</u> of <u>on-beta ganglion cells</u> have been measured in a <u>perfused cat eye cup</u>. Dark resistances were typically about 45 megohms, and all components of the light evoked response produced a small decrease in resistance. <u>Reversal potentials</u> for depolarizing response components lay positive to resting levels, but between values typical of ionic Nernst potentials, suggesting that both <u>excitatory</u> and <u>inhibitory ionic drives</u> were simultaneously active. <u>Off hyperpolarization</u> reversed <u>negative</u> to the resting potential. The results relate to <u>electron microscopic</u> studies of synaptic impingement onto on-beta cells.		
4 LNP, DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02705-02 LNP
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Anatomical Studies of Neurons, Neurotransmitters and Neurotransmitter Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: A.P. Mariani, Special Expert, LNP, DIR, NINCDS. Others (LNP, DIR, NINCDS): J.L. Barker, Chief; J.N. deMelo, Visiting Associate and N.L. Silva, NRC Fellow		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 2.0	PROFESSIONAL 2.0	OTHER 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The autofluorescent serotonin analog, 5,7 dihydroxytryptamine, has been used to identify <u>living dopaminergic neurons</u> derived from the <u>mesencephalon</u> . Midbrains of embryonic day 13 rats were grown in vitro for three weeks, incubated with micromolar amounts of 5,7 dihydroxytryptamine and examined by fluorescence microscopy. When excited by ultraviolet light (wavelength max. 365nm) a small proportion of the neurons displayed a weak, but well defined, <u>blue-violet fluorescence</u> . The neurons so identified were photographed and marked and subsequently fixed in aldehydes to be processed for aldehyde-induced (Faflu) catecholamine fluorescence or tyrosine hydroxylase <u>immunohistochemistry</u> . Recovery of the identified neurons showed that 100% displayed catecholamine fluorescence and over 80% were immunoreactive for tyrosine hydroxylase. These experiments demonstrate that uptake of the fluorescent serotonin analog 5,7 dihydroxytryptamine can be used to identify living dopaminergic neurons derived from the midbrain. This approach will now allow focussed single cell analyses which otherwise would be at best most difficult since dopaminergic neurons constitute one of the rarest <u>transmitter phenotypes</u> in the CNS.		
5 LNP, DIR		

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Molecular and Cellular Neurobiology

National Institute of Neurological and Communicative Disorders and Stroke

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October 1, 1986 through September 30, 1987

Laboratory of Molecular and Cellular Neurobiology

National Institute of Neurological and Communicative Disorders and Stroke

Richard H. Quarles, Ph.D., Chief (Rotating)

The Laboratory of Molecular and Cellular Neurobiology (LMCN) was formed in Fiscal Year 1987 by combining four Sections that were previously part of other Laboratories or Branches in NINCDS. The four Sections have complementary research interests with a strong basis in biochemistry, and the position of Laboratory Chief will rotate annually among the four Section Chiefs. It is anticipated that combining of the four established Sections will result in a strong new Laboratory with a critical intellectual mass sufficient to offset the disadvantage of the off-campus site in the Park Building in Rockville, Maryland where the LMCN will be located for at least the next five years.

The four Sections making up the new Laboratory include: the Membrane Biochemistry Section, headed by Dr. Peter Fishman and previously part of DMNB; the Myelin and Brain Development Section, headed by Dr. Richard Quarles and also previously part of DMNB; the Neural Systems Section, headed by Dr. Daniel Alkon and previously located at Woods Hole, MA as part of LB; and the Receptor Biochemistry and Molecular Biology Section, headed by Dr. Craig Venter and previously part of LNP.

The LMCN conducts research on the structure and function of neural tissue with emphasis on membrane biochemistry, myelin, receptors, and molecular and biophysical mechanisms of learning and memory. The research is multidisciplinary including biochemistry, biophysics, immunology, molecular biology, neurophysiology and cell biology. Molecules under investigation include complex glycolipids, ion channels, cell surface adhesion glycoproteins, myelin proteins, and receptors for neurotransmitters, hormones, and toxins. The research is relevant to neurological diseases and disorders of behavior.

The Membrane Biochemistry Section investigates the structure and metabolism of cell membrane components with emphasis on the functional roles of cell surface glycoconjugates. Specific goals of the research include elucidation of molecular mechanisms involved in transmembrane signaling of receptor mediated information, topographical organization of cell surface receptors into functional domains, and membrane involvement in neurological, neoplastic and endocrine disorders. The Myelin and Brain Development Section investigates the biochemistry of myelin and other membranes in the nervous system, with emphasis on glycoproteins and glycolipids and their roles in cell surface phenomena during development. The Section also investigates molecular aspects of the pathogenesis of multiple sclerosis and demyelinating diseases of the peripheral nervous system. The Neural Systems Section investigates biophysical and molecular mechanisms of associative learning in parallel for a mollusc (sea snail, Hermisenda) and a mammal (rabbit). Ultimate goals of this research are to arrive at clinically meaningful interventions and to design artificial intelligence with associative memory capability. The Receptor Biochemistry and Molecular Biology Section conducts research on neurotransmitter receptor proteins to determine structure, immunological properties, molecular mechanism of function, regulation of expression and evolutionary aspects. The Section also investigates the role of receptor structure, immunology and regulation in receptor related disorders.

A more detailed summary of the recent research and especially important progress made in the past year are given below for three of the four Sections. The report for the Membrane Biochemistry Section is still included with LMNB since that Section did not actually move to the Park Building in Fiscal Year 1987 and still functions in close proximity to LMNB.

Section on Myelin and Brain Development

1. Structure and function of the myelin-associated glycoprotein (MAG).

Previous immunocytochemical studies had shown that MAG is selectively localized in periaxonal Schwann cell or oligodendroglial membranes of myelin sheaths suggesting that this glycoprotein functions in the formation and maintenance of the junction between these myelin-forming cells and the axolemma. This hypothesis was strongly supported by studies on dysmyelinating quaking mice showing a correlation between the absence of MAG in the periaxonal Schwann cell membranes and the breakdown of the normal 12-14 nM space between the inner Schwann cell membrane and the axolemma. Based on these findings a molecular model was proposed to indicate how MAG might be involved in the maintenance of this glial-axonal junction. MAG is also present in Schmidt-Lantermann incisures, lateral loops and the outer mesaxons of PNS myelin sheaths and is believed to play a role in maintaining the spacing of adjacent Schwann cell membranes in these structures where extracellular membrane surfaces are separated by 12-14 nM and there is always cytoplasm at the inner membrane surface. A major advance in the past year has been elucidation of the complete amino acid sequence of MAG from the characterization of full length cDNA clones from rat brain. This information has revealed various properties of MAG that are similar to those proposed in our molecular model and strongly support and elaborate upon the above functional hypothesis. MAG contains a single transmembrane domain separating a large heavily glycosylated N-terminus from an intracellular C-terminal tail with potential phosphorylation sites that could affect its interaction with cytoskeletal elements. In fact, there are two developmentally regulated forms of MAG generated by alternative splicing of the primary mRNA transcript that have different C-terminal tails. This finding explains the previous results from our laboratory showing a decrease in the apparent molecular weight of MAG during development. Of particular interest is the finding that the N-terminus of MAG consists of five domains related in amino acid sequence to each other and to various members of the immunoglobulin superfamily including neural cell adhesion molecule (N-CAM). Thus MAG appears to be a member of a subgroup of glycoproteins that are in the immunoglobulin superfamily and function in cell-cell interactions in the nervous system. Another aspect of MAG structure that may be relevant to its role in cell-cell interactions is an Arg-Gly-Asp sequence which is known to be a binding site by which a growing number of extracellular proteins attach to cell surfaces. Still another similarity of MAG to N-CAM and other brain glycoproteins involved in adhesion is the presence of the carbohydrate structure recognized by HNK-1 and related antibodies. Overall, the MAG molecule seems well suited to mediate interactions between intracellular cytoskeletal elements and adjacent extracellular membrane surfaces. In this manner, MAG could play a key role in the chemo-mechanical forces involved in generating the spiraled myelin sheaths.

2. Dysmyelination. Quantitative studies of MAG and other myelin proteins in CNS tissue from lambs with Border Disease, a dysmyelination caused by a pestivirus, gave results similar to those obtained previously in various dysmyelinating genetic mutants. It was found that myelin basic protein (MBP) and proteolipid protein (PLP) were reduced more than MAG or 2'3'-cyclic

nucleotide 3'-phosphohydrolase (CNPase), presumably because the affected lambs have a greater deficiency of compact myelin than of associated oligodendroglial membranes in which MAG and CNPase are concentrated. The findings suggest that the pestivirus affects myelin expression by oligodendrocytes in a manner generally similar to the genetic lesions.

In view of recent interest in the possible involvement of the PLP gene that is on the X-chromosome in sex linked dysmyelinating mutants, PLP expression was examined in the X-linked canine shaking pup mutant. Similarly to previous findings in X-linked jimpy mice and myelin deficient rats, PLP was reduced more than the other myelin proteins. Interestingly, the related DM-20 protein formed by alternative splicing of the primary PLP mRNA transcript was expressed in amounts equal to or greater than PLP, although normal animals always express about 10 fold more PLP than DM-20. The greater deficit of PLP than other myelin proteins and the disproportional expression of DM-20 and PLP suggest that the PLP gene is affected directly in this X-linked mutation.

3. MAG in multiple sclerosis. Previous immunocytochemical and quantitative biochemical studies had demonstrated a greater loss of MAG than other myelin proteins at the periphery of multiple sclerosis plaques suggesting an important role for MAG in the molecular pathology of this disease. More recent studies demonstrated that much of the MAG in affected areas of MS brain is in the form of a proteolytic derivative of MAG, dMAG, that is formed by the action of a myelin-associated, Ca-activated neutral proteinase (CANP). This enzyme is more active in myelin from MS brain than from control brain. In the past year using antibodies specific for the C-terminus of the MAG molecule, we have demonstrated that dMAG results from the proteolytic removal of much of the C-terminal tail. dMAG is not as tightly associated with the membranes of the myelin sheath as intact MAG, and a substantial amount of it appears in the cerebrospinal fluid. A likely explanation for the preferential loss of MAG from the periphery of MS plaques is its conversion to dMAG and release into extracellular fluids, possibly leading to a disruption of glia-axon interactions.

4. Peripheral neuropathies. More than half of patients with peripheral neuropathy occurring in association with IgM paraproteinemia have a monoclonal antibody that reacts with a carbohydrate epitope that is in MAG, some 20 to 26 kDalton glycoproteins of peripheral nerve myelin, and sulfate-3- glucuronyl paragloboside (SGPG). Although the antibodies from all patients of this type are similar in binding to the same group of glycoconjugates, studies on the affinity of antibody binding to each of the glycoconjugates and to derivatives of SGPG have revealed idiotypic heterogeneity among patients with regard to the precise chemical requirements for antibody binding. Studies in the past year have also shown that intraneural injection of cats with serum from these patients supplemented with complement produces extensive demyelination. Although this result strengthens the case for the monoclonal antibody causing the neuropathy in humans, the relationship of the antibody to the pathology is not yet entirely clear for several reasons. For example, complement is not generally detected in biopsies of the human patients' nerves, and the demyelination occurring after intraneural injection of cat nerve is inflammatory while the lesions in human nerves are noninflammatory.

In patients with IgM paraproteinemia and neuropathy in which the monoclonal antibody does not react with MAG, we have found that the antibody often reacts with ganglioside antigens. Ganglioside antigens that have been

detected include GM1, GM2, GD3, GD1b and GT1b. Overall, since anti-MAG paraproteins also react with SGPG, we have found that more than 80% of monoclonal IgM antibodies in patients with neuropathy react with acidic glycolipid antigens. This suggests that acidic glycolipids may be important target antigens in the paraproteinemic neuropathies.

Since antibodies to acidic glycolipid antibodies are common in the paraproteinemic neuropathies, we have also looked for antibodies with this specificity in the clinically more prevalent inflammatory neuropathies. High titers of antibodies to gangliosides were detected in 5 of 26 patients with Guillain-Barre syndrome and in 2 of 17 patients with chronic inflammatory demyelinating polyneuropathy. When longitudinal serum samples were available for study, it was found that antibody titers were high at the time of disease onset and fell coincidentally with clinical improvement. The specificities of the antibodies were different in most cases, but similar antibodies were not found in a series of other neurological disease controls or in healthy subjects. The frequency of occurrence of the ganglioside antibodies in the inflammatory neuropathies was significantly greater than in the combined controls at $p < 0.01$. The results suggest that antibodies to gangliosides could be involved in the pathology of some inflammatory neuropathies as well as paraproteinemic neuropathies.

Section on Neural Systems

The Section on Neural Systems takes a multidisciplinary approach to the question of how information is stored during associative learning and how it is made available for later recall. Biophysical and molecular mechanisms of associative learning are being analyzed in parallel for a mollusc (the sea snail Hermissenda) and a mammal (the rabbit). Parallel analyses offer the important opportunity for uncovering general cellular principles of learning and memory - principles which have been conserved over the course of evolution and which therefore could have relevance for human cognition. Parallel analyses also permit exploitation of critical experimental advantages unique to these two species. For Hermissenda we have been able to demonstrate the first casual relationship of biophysical and molecular transformations within individual neurons to Pavlovian conditioning of a living animal. Casual relationships of cellular physiology and associative learning have not yet been approximated for any vertebrate preparation. Nevertheless we found evidence of biophysical transformations which are common to both mollusc and mammal. An identified group of neurons, the CAL cells (rather than individual identified neurons) was shown to have a distribution of conditioning-specific modification of K^+ channels within hippocampal slices removed from rabbits on days after they had been conditioned. Such slices provide vastly greater amounts of tissue (than does the snail) for biochemical studies. Indeed, we have already observed conditioning-specific translocation of C-kinase not only in the hippocampus CAL neurons, but also in a restricted region of the cerebellar cortex called "H6". C-kinase regulation of identical K^+ channels occurs in both Hermissenda and hippocampal neurons. In Hermissenda this regulation is being pursued with isolated membrane patches whose intracellular surfaces are accessible to precise ionic and biochemical manipulations. Most recently, C-kinase regulation has been linked to conditioning-specific modification of Hermissenda m-RNA metabolism. This molecular storage step occurs within a specific temporal window during the retention period of the associative memory. It may represent an important step for consolidating, i.e., making more permanent the physiologic memory trace which could have expression in

conditioning-specific structural changes of Hermisenda neurons. These latter changes, also only uncovered during the last year, involve an apparent reorganization of the cell's terminal branches on which synaptic interactions occur.

The experimental psychology program of the Section uses associative learning paradigms to produce persistent behavioral changes in the nudibranch mollusc Hermisenda crassicornis as well as vertebrate species such as rabbits. Quantitative assessments are made of the animals' responses to the conditioned and unconditioned stimuli before and after classical conditioning paradigms. These assessments include precise dissection of generalized behavioral transformations into modification of individual muscular components of the behaviors. A full range of psychological manipulations have been used to clearly establish the sensitivity of the learning behavior to the exact temporal relationship of the stimuli which are associated during acquisition of the learning. Also of interest to the psychologists is the close linkage of the learning behavior to the specific stimuli associated and discriminative functions involving those stimuli not associated.

The Section's neurophysiology program is concerned first with the definition of those neural systems relevant to the learning capability. Multiple intracellular recordings from pre- and postsynaptic neurons have been employed within the visual, vestibular and chemosensory pathways of Hermisenda to establish a working knowledge of the critical neural systems and to describe how information flows in a stepwise fashion beginning with the sensory cells at the output. A similar approach is being taken with the rabbit hippocampus, and critical afferent and efferent pathways within this structure. Neurophysiological correlates are then obtained (again for both the mollusc and the rabbit) for conditioned (as well as a variety of control) animals. These neurophysiological correlates are recorded in intact animals, isolated nervous systems, and isolated neuronal membranes. Based on such correlates electrophysiological sequences are constructed to trace the transformation of the information in electrical terms of the neural system.

The Section's biophysics program measures persistent modification of specific ionic channels during and following the learning. In the past, a two microelectrode voltage clamp was employed to characterize genetically specified membrane currents within identified neurons which were demonstrated to play a casual role in the acquisition and retention of associative learning. More recently the patch-clamp technique has been used in both the cell-attached and "inside-out" configurations to determine which subcellular biochemical processes (e.g., Ca^{2+} -dependent phosphorylation) are critical for regulating those ionic channels which change during learning. All of these biophysical approaches have also been applied to unequivocally demonstrate that it is in fact persistent modification of specific ionic channels which encode a learned association for later recall.

The biochemistry research of the Section seeks to uncover the molecular basis for the persistent ionic channel modifications shown to underlie associative learning (both in Hermisenda and the rabbit). A variety of biochemical and molecular biological methods are being brought to bear for this purpose. Microgel analysis of phosphorylation of individual neuronal proteins, for example, has revealed that Ca^{2+} -dependent phosphorylation of a specific low-molecular weight protein changes within certain neurons of conditioned animals but not those exposed to control paradigms. Exposure of neurons to prolonged depolarization, which simulates the integrated visual-vestibular

network effects on identified neurons during conditioning, is also followed by long-lasting phosphorylation differences for particular low molecular weight proteins. Furthermore, a number of intracellular manipulations have provided support for the hypothesis that learning-induced modification of ionic channels involves Ca^{2+} -calmodulin-dependent and Ca^{2+} -and lipid-dependent phosphorylation. Such manipulations include iontophoretic injection of Ca^{2+} -calmodulin-dependent protein kinase (type II brain), inositol trisphosphate or phosphatase, or preincubation with C-kinase activators such as phorbol esters or OAG. Modern molecular biological techniques are also now making available for the Section's use monoclonal antibodies to enzymes (e.g., the type II kinase) implicated in the learning process. Other antibodies (to phosphatase) may also prove helpful for our reconstruction of the biochemical and associated biophysical sequences which make biological records of memory possible. Recombinant techniques may be particularly helpful in identifying specific proteins whose synthesis is modified as a result of the recently observed conditioning-specific increase in m-RNA turnover.

The cellular anatomy aspect of the Section's programs contributes in several ways to the various levels of inquiry into the learning process already mentioned. Ultrastructural measurements of the cells and their synaptic interaction has provided further definition of the relevant neural systems. Activity-dependent uptake of radioactive labels within these systems has been monitored by autoradiographic methods. Morphometric techniques together with serial sectioning and computerized reconstruction, have recently uncovered structural manifestations of the biophysical and biochemical changes already shown for neurons within conditioned but not control animals. Differential absorption spectrophotometry allows intracellular localization of fluctuations of cytosolic Ca^{2+} -dependent modulation of the channels during learning.

Finally, the developmental biologists within the Section have established laboratory strains of Hermisenda. Such strains permit assessment of how genetic and environmental factors may interact to determine individual differences in the ability of the animals to undergo associative learning.

Perhaps most important in all of these efforts is the accumulated evidence that a remarkable similarity exists between means of encoding learned associations in the snail and the rabbit. The same learning-induced reduction of well-characterized K^+ currents has been found to provide such encoding in Hermisenda as it does within identified neurons of rabbit hippocampal slices. Similar regulation of these channels appears to occur at the molecular level for both the mollusc and the mammal. Such parallel mechanisms may ultimately provide the basis for clinical intervention and thereby the amelioration of pathologic symptoms.

Section on Receptor Biochemistry and Molecular Biology

The long term objectives of the Section of Receptor Biochemistry and Molecular Biology are to elucidate the structure, function and evolution of the neurotransmitter receptors of the autonomic nervous system. These receptors include muscarinic cholinergic receptors and alpha- and beta-adrenergic receptors. A combination of complementary techniques have been and are currently being utilized in the study of receptor structure and function. These include receptor purification using affinity chromatography, isoelectric focusing, ion exchange chromatography, SDS-polyacrylamide gel electrophoresis and HPLC; production of monoclonal and polyclonal antibodies to intact receptor

proteins as well as to synthetic peptides corresponding to distinct receptor domains; cloning and sequence analysis of genes encoding neurotransmitter receptor proteins from a number of species; site-directed mutagenesis of receptor genes to localize residues important in receptor function and the use of DNA and RNA probes to study receptor distribution, localization and regulation.

Biochemical and immunological studies from the Section of Receptor Biochemistry and Molecular Biology during the past three years have indicated that the receptors of the autonomic nervous system share a high degree of structural homology. Specifically, β_1 and β_2 -adrenergic receptors appear to be very closely related proteins that are identical in terms of the ability to stimulate adenylate cyclase, size on SDS-PAGE, isoelectric points and monoclonal antibody cross-reactivities. Similarly, α_1 and α_2 -adrenergic receptors appear to be related "isoreceptors" that share a number of gross biochemical properties. Data obtained from monoclonal antibody studies also suggested that muscarinic cholinergic and α -adrenergic receptors shared regions of structural homology.

During the past year we have expanded the program of the Section and have begun an intensive effort on the cloning and sequence analysis of neurotransmitter receptor genes. To date, we have obtained the nucleotide and deduced amino acid sequences of the β -adrenergic receptors from human brain, rat heart and rat adipose tissue and of muscarinic cholinergic receptors from rat heart and brain. In addition, we are now involved in the cloning and sequence analysis of neurotransmitter receptor genes from lower species including blue and white sharks, Drosophila, Hermisenda and Limulus. These studies taken together with other reports on the sequence of autonomic receptor genes indicate that the adrenergic and muscarinic cholinergic receptors are members of a multi-gene family and share considerable primary sequence homology among species and between the major receptor subclasses. These data confirm and extend our previously published findings on the relationship between these receptor proteins.

We are also devoting a major effort to the high density expression of cloned neurotransmitter receptor genes in cells lacking these proteins. To date, we have produced several stable clonal murine cell lines expressing human β -adrenergic receptors at a density approximately 20 fold higher than that found in most cell types. Ligand binding analysis has revealed that the receptor encoded by this gene is a β_2 -adrenergic subtype. The gene appears to contain all of the information necessary for the normal processing and functional expression of the human β -receptor. The availability of stable cell lines expressing human neurotransmitter receptors offers a potentially useful tool for the design and screening of new therapeutic agents. In addition, these cell lines offer a new source of material for large scale receptor purification and crystalization.

A logical extension of the work on receptor gene expression involves site-directed mutagenesis studies to elucidate the amino acid residues involved in such receptor functions as ligand binding and signal transduction to coupling proteins. We are currently studying a large number of β -adrenergic receptor site-directed mutants in order to identify the site(s) of agonist and antagonist binding and kinase mediated receptor phosphorylation which has been implicated in receptor desensitization.

The availability of cDNA probes for neurotransmitter receptors allows for the study of receptor regulation under a variety of conditions. We have characterized the glucocorticoid induction of beta-adrenergic receptors in a line of cultured human lung cells and have demonstrated that the approximate doubling of receptor density following exposure of cells to steroid is preceeded by a parallel increase in the level of receptor specific messenger RNA. These studies suggest that glucocorticoid regulation of beta-adrenergic receptor density occurs at the level of gene transcription. We are now also involved in similar studies examining the effects of thyroid hormone on receptor densities in various tissues in the rat.

In collaboration with Richard Feldman of DCRT, we have undertaken detailed studies of the secondary and tertiary structure of adrenergic and cholinergic receptors by computer modeling. These studies in conjunction with the site-directed mutagenesis experiments are particularly exciting as they could define the ligand binding sites of these receptors and allow for the accurate prediction of new drug structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01808-18 LMCN																				
PERIOD COVERED October 1, 1986 through September 30, 1987 *																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Glycoproteins of Myelin in Development and Disease																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Richard H. Quarles, Ph.D.</td> <td style="width: 20%;">Section Chief</td> <td style="width: 25%;">LMCN, NINCDS</td> </tr> <tr> <td rowspan="5">Others:</td> <td>Daniel O'Shannessy, Ph.D.</td> <td>Visiting Associate</td> <td>LMCN, NINCDS</td> </tr> <tr> <td>Antonio Noronha, Ph.D.</td> <td>Visiting Fellow</td> <td>LMCN, NINCDS</td> </tr> <tr> <td>Hugh Willison, M.D.</td> <td>Visiting Fellow</td> <td>LMCN, NINCDS</td> </tr> <tr> <td>Johanna Moller, M.D.</td> <td>Visiting Fellow</td> <td>LMCN, NINCDS</td> </tr> <tr> <td>Hiroko Baba, M.D.</td> <td>Visiting Fellow</td> <td>LMCN, NINCDS</td> </tr> </table>			PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN, NINCDS	Others:	Daniel O'Shannessy, Ph.D.	Visiting Associate	LMCN, NINCDS	Antonio Noronha, Ph.D.	Visiting Fellow	LMCN, NINCDS	Hugh Willison, M.D.	Visiting Fellow	LMCN, NINCDS	Johanna Moller, M.D.	Visiting Fellow	LMCN, NINCDS	Hiroko Baba, M.D.	Visiting Fellow	LMCN, NINCDS
PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN, NINCDS																			
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	Hugh Willison, M.D.	Visiting Fellow	LMCN, NINCDS																			
	Johanna Moller, M.D.	Visiting Fellow	LMCN, NINCDS																			
	Hiroko Baba, M.D.	Visiting Fellow	LMCN, NINCDS																			
COOPERATING UNITS (if any) Scripps Clinic, La Jolla, CA; Department of Neurology, Johns Hopkins Univ., Balto., MD; Laboratory of Molecular Genetics, NINCDS; Infectious Diseases Branch, NINCDS; Univ. Wisconsin Veterinary School, Madison, WI																						
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology																						
SECTION Section on Myelin and Brain Development																						
INSTITUTE AND LOCATION Park Building, Rm. 425, NINCDS, NIH, Bethesda, MD 20892																						
TOTAL MAN-YEARS 8.1	PROFESSIONAL: 5.6	OTHER: 2.5																				
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) The <u>myelin-associated glycoprotein (MAG)</u> is localized in the periaxonal membranes of PNS and CNS <u>myelin sheaths</u> where it appears to be involved in <u>glia-axon interactions</u>. Structural information about MAG obtained from sequencing of <u>cDNA clones</u> indicates that it has a single transmembrane domain separating two different <u>developmentally regulated</u> C-terminal tails generated by <u>alternative splicing</u> of the primary mRNA transcript from a heavily glycosylated N-terminus containing five domains that are related in sequence to each other and to molecules in the <u>immunoglobulin gene superfamily</u> such as <u>neural cell adhesion molecule</u>. In the <u>dysmyelinating shaking dog mutant</u>, the combined amount of <u>proteolipid protein (PLP)</u> and the related <u>DM-20 protein</u> are reduced to less than 2% of the control level and are disproportionately expressed in comparison to controls, suggesting that the PLP gene is the site of the primary genetic lesion in this <u>X-linked mutation</u>. In <u>multiple sclerosis</u>, MAG is reduced more than PLP or <u>myelin basic protein</u> in periplaque areas, and in many lesions much of the MAG is in the form of its <u>proteolytic derivative, dMAG</u>. Further studies on human <u>IgM paraproteins</u> associated with <u>neuropathy</u> revealed <u>idiotypic heterogeneity</u> among the antibodies from different patients with regard to their binding to <u>sulfate-3- glucuronyl paragloboside</u> and MAG. Injection of cat nerves with fresh sera from these patients supplemented with complement caused extensive <u>demyelination</u> adding to the evidence that the anti-MAG antibodies cause the neuropathy in these patients. A high proportion of patients with <u>IgM gammopathy</u> and neuropathy have paraproteins that react with various gangliosides, indicating that <u>acidic glycolipid antigens</u> are common in this type of neuropathy. High levels of antibodies to various gangliosides were found in about 15% of patients with <u>Guillain-Barre syndrome</u> and <u>chronic demyelinating inflammatory polyneuropathy</u>, a frequency significantly higher than in normal or other neurological disease controls. 9 LMCN/DIR [*Transferred from DMN on 7/87]																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02151-13 LMCN
PERIOD COVERED October 1, 1986 through September 30, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Information Processing in Simple Nervous Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: D.L. Alkon Medical Officer LCMN NINCDS Others: C. Collin Visiting Fellow LCMN NINCDS J. Disterhoft IPA Fellow LCMN NINCDS M. Kubota Visiting Fellow LCMN NINCDS A. Kuzirian Staff Fellow LCMN NINCDS S. Naito Special Expert LCMN NINCDS H. Rasmussen Guest Worker LCMN NINCDS T. Nelson Staff Fellow LCMN NINCDS	J. Cosgrove Staff Fellow, LCMN J. Blaszczyk Visiting Fellow LCMN NINCDS	
COOPERATING UNITS (if any) MBL, Woods Hole, MA 02543 (J. Harrigan, I. Lederhendler, D. McPhie, H. Ikeno, J. LoTurco); Boston University Marine Program (C. Chen; D. Coulter); Medical Research Council, Canada (B. Bank); Deutsche Forschungs. (H.-P. Hopp)		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology		
SECTION Section on Neural Systems		
INSTITUTE AND LOCATION Park Building, Rm. 425 NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 10.0	PROFESSIONAL: 9.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The principal objective of the program is to define molecular and biophysical mechanisms of learning and memory. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals of such research are to arrive at clinically meaningful interventions on the one hand, and, to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principle frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than non-associative behavioral modifications (such as sensory adaptation, habituation, arousal and sensitization). The <u>biological basis of learning and memory</u> is of interest at several levels of complexity: <u>behavioral phenomena, neuronal systems, neuronal membranes, and molecular transformations</u>. To literally reconstruct the physiology involved (and then to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusc <u>Hermisenda crassicornis</u> as well as "complex system" preparations such as rabbits. The mollusc can work thus far has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of <u>identified single neurons</u> it has proven possible to define biochemical pathways which regulate such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations have been shown in our program to record associative memory in the rabbit as were found in <u>Hermisenda</u>. Rabbit neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., C-kinase) pathways which control membrane excitability have recently been demonstrated. (*Transferred from LB on 7/87)		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02670-03 LMCN
PERIOD COVERED October 1, 1986 to September 30, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evolution of Neurotransmitter Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.C. Venter, Section Chief, LMCN, DIR, NINCDS. Others (LMCN, DIR, NINCDS): C.M. Fraser, Research Physiologist; F.-Z. Chung, Staff Fellow A. Kerlavage, Senior Staff Fellow; D. Robinson, Biologist; J. Lai, Fogarty Fellow; K.-U. Lentes, Guest Researcher; J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow; M. Fitzgerald, Biologist; S. Arakawa, Fogarty Fellow; T. Onai, Guest Researcher.		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology, DIR, NINCDS		
SECTION Section on Receptor Biochemistry and Molecular Biology		
INSTITUTE AND LOCATION Park Bldg., Rm. 405, NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this project is to examine the structural and functional evolution of neurotransmitter receptor proteins. Using muscarinic cholinergic and beta adrenergic receptors as the initial model systems we have isolated and characterized these receptors from the brains and hearts of a wide variety of species. Brain and heart tissue have been obtained from all vertebrate classes including mammals, birds, reptiles, amphibians, and fish as well as from lower species including insects (<i>Drosophila</i>). Data from muscarinic receptor studies demonstrate that out of all of the parameters studied (SDS gel molecular weight, isoelectric point, monoclonal antibody cross reactivity, agonist affinity, antagonist affinity, stereospecificity of ligand binding, GTP shifts of agonist affinity, and receptor density) only receptor density appears to have changed over 900 million years of evolution gradually increasing in higher species. Muscarinic receptors have been purified from human brain, rat brain and heart, pig heart and shark brain and heart in order to compare primary structures in detail. The evolutionary relationship between muscarinic cholinergic receptors and other neurotransmitter receptors is also being studied. Protein sequence data and defined gene probes are being employed to follow the evolution of adrenergic, cholinergic and GABAergic receptors. We have cloned and sequenced beta and muscarinic genes from human and rat and have isolated a number of clones from other species. In particular, a positive clone from <i>Drosophila</i> genomic library has been isolated. Partial sequence data reveals that this clone contains a stretch of fifty-four nucleotides which has 84 homology with the corresponding human beta₂ sequence. When the sequence is translated into amino acids and favored substitutions are considered, the homology goes up to 100. Although the exact nature of this beta₂-like gene is still unknown, it may provide valuable information toward our understanding of the evolution of neural receptors. * (Transferred from LNP on 7/87).		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02671-03 LMCN
PERIOD COVERED October 1, 1986 to September 30, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunological Characterization and Localization of Neurotransmitter Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: C.M. Fraser, Research Physiologist, LMCN, DIR, NINCDS. Others (LMCN, DIR, NINCDS): J.C. Venter, Section Chief; D. Robinson, Biologist; J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow		
COOPERATING UNITS (if any) George N. McNeil, Maine Medical Center, Portland, Maine		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology, DIR, NINCDS		
SECTION Section on Receptor Biochemistry and Molecular Biology		
INSTITUTE AND LOCATION Park Bldg., Rm. 405, NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The aims of this research are to develop extensive libraries of polyclonal and monoclonal antibodies to major classes of neurotransmitter receptors including muscarinic cholinergic, alpha and beta-adrenergic, GABAergic and opiates; and to utilize these reagents in studies of receptor structure and function, the degree of molecular homology between receptor subclasses, the localization of neurotransmitter receptors on cell membranes, the evolution of receptor subclasses and the role of anti-receptor antibodies in human diseases. Principal observations to date include the following: 1) monoclonal antibodies raised against each major class of neurotransmitter receptor recognize the same receptor in a number of different tissues and species suggesting considerable conservation of receptor structure throughout evolution; This observation has been borne out with recent data on the nucleotide sequence encoding these receptors. 2) certain monoclonal antibodies raised against muscarinic cholinergic receptors recognize alpha₁-adrenergic receptors and vice versa suggesting structural homology between these pharmacologically distinct receptor sub-types; 3) monoclonal antibodies raised against alpha₁-adrenergic receptors recognize alpha₂-adrenergic receptors, and this information taken together with pharmacological and biochemical data suggests that alpha₁- and alpha₂-adrenergic receptors may be closely related "isoreceptors"; 4) monoclonal antibodies to muscarinic cholinergic receptors have been utilized to fluorescently label receptors on the surface of cultured cells and in brain sections demonstrating the potential usefulness of these reagents in studies of receptor localization and distribution in various species and tissues as well as in studies of receptor processing and turnover; and 5) some patients suffering from depression possess autoantibodies to human brain membrane proteins. The identity of these proteins is being pursued.* (Transferred from LNP on 7/87). 12 LMCN/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02672-03 LMCN
PERIOD COVERED October 1, 1986 to September 30, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurotransmitter Receptor Purification and Structure		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: J.C. Venter, Section Chief, LMCN, DIR, NINCDS. Others (LMCN, DIR, NINCDS): C.M. Fraser, Research Physiologist; A. Kerlavage, Senior Staff Fellow; D. Robinson, Biologist; J. Lai, Fogarty Fellow; K.-U. Lentes, Guest Researcher, J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow; M. Fitzgerald, Biologist		
COOPERATING UNITS (if any) Richard J. Feldmann, Division of Computer Research and Technology, NIH		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology, DIR, NINCDS		
SECTION Section of Receptor Biochemistry and Molecular Biology		
INSTITUTE AND LOCATION Park Bldg., Rm. 405, NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 3.0	PROFESSIONAL: 2.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Neurotransmitter receptors; adrenergic (beta₁, beta₂, alpha₁, and alpha₂), cholinergic (muscarinic and nicotinic), and benzodiazepine receptors are being isolated and purified in order to understand the molecular basis of receptor function and neuronal communication. Specific projects are underway to provide precise structural information on each of the above receptor proteins. Structural data being obtained include primary sequence data, proteolytic digest maps, topology information and structure-function data, e.g., neurotransmitter binding site localization, sugar localization, membrane domain and effector coupling protein recognition domains. Our data have demonstrated that structural similarities exist among non-pharmacologically related neurotransmitter receptors (muscarinic, cholinergic and alpha adrenergic) and that these neurotransmitter receptors mediate cellular modulation via protein conformational changes initiated by neurotransmitter binding to the binding site in their transmembrane domains. Receptor coupling is mediated by the intracellular loops of the receptors which appears to be the effector protein (GTP-regulatory protein) recognition portion of the receptor. Electron microscopy and high performance size-exclusion chromatography of purified receptors indicate that the alpha₂ and beta₂ receptors exist as homodimers while the muscarinic receptor is monomeric. Protein preparative procedures have been established which include various HPLC steps, ligand affinity chromatography, monoclonal antibody affinity chromatography, preparative SDS-gel electrophoresis, lectin affinity chromatography, ion exchange purification protocols is now permitting simultaneous detailed structural comparisons of all adrenergic and cholinergic receptor proteins as well as proteins expressed from cloned receptor genes. *(Transferred from LNP on 7/87).</u>		
13 LMCN/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02710-02 LMCN
PERIOD COVERED October 1, 1986 to September 30, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Neurotransmitter Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: J.C. Venter, Section Chief, LMCN, DIR, NINCDS. Others(LMCN, DIR, NINCDS) F.-Z. Chung, Staff Fellow; C.M. Fraser, Research Physiologist; K.-U. Lentes, Guest Researcher; J. Lai, Fogarty Fellow; D.A. Robinson, Biologist; J.D. Gocayne, Biologist; P.C. Potter, Fogarty Fellow; A.R. Kerlavage, Senior Staff Fellow; M.G. Fitzgerald, Biologist; S. Arakawa, Fogarty Fellow; T. Onai, Guest Researcher; Cheng-Dian Wang, Guest Researcher.		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology, DIR, NINCDS		
SECTION Section of Receptor Biochemistry and Molecular Biology		
INSTITUTE AND LOCATION Park Bldg., Rm. 405, NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 6.0	PROFESSIONAL 5.0	OTHER 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Recently, we published a paper on Cloning and Sequence Analysis of the Human Brain beta-Adrenergic Receptor (FEBS Lett. 211:200-206, 1987). In a recent review concerning the evolution of adrenergic and cholinergic receptors we further postulated that the muscarinic cholinergic receptor has existed for over 600 million years (Prog. Neurobiol., in press). Pharmacological and biochemical evidence suggests that the beta-adrenergic receptor appeared later in evolution raising the possibility that it evolved as a result of gene duplication. In order to further answer questions concerning receptor evolution we are in the process of cloning beta-adrenergic and muscarinic cholinergic receptors from shark and <u>Drosophila</u> tissue. The cloning of neural receptor genes also allowed us to study the structure-function relationship of these receptors by site-directed mutagenesis. We have successfully expressed the human beta ₂ -adrenergic receptor gene in a mouse cell line previously lacking the functional receptor. A series of single amino acid substitution mutants have also been made and are in the process of being characterized. Study of these mutants should help us to locate those important residues responsible for ligand binding, signal transduction or maintenance of structural integrity. *(Transferred from LNP on 7/87).		
14 LMCN/DIR		

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Laboratory of Neuronal Growth and Regeneration
Division of Intramural Research

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987

Laboratory of Neuronal Growth and Regeneration

National Institute of Neurological and Communicative Disorders and Stroke

Andrew A. Zalewski, M.D., Acting Chief

The Laboratory of Neuronal Growth and Regeneration (LNGR) was established in FY 1987. The objectives of this laboratory are: 1) to conduct research on neuronal growth and its controlling factors and on axonal regeneration, either spontaneous or after the implantation of other neurons or grafted tissue; and 2) to develop experimental animal models of human disease and explore physiological, biochemical, and pharmacological methods to assess changes in neuronal function and activity attending the disorder; the methods are then used to evaluate attempts to correct the disorder by promotion of regeneration or growth on nerve tissue implants.

Experiments will be conducted on both the central and peripheral nervous systems. There are currently two Sections in the LNGR; Neuronal Development and Regeneration; and Neuropharmacology.

The Section on Neuronal Development and Regeneration has continued to evaluate the feasibility of using foreign nerve grafts to repair injured peripheral nervous tissue. Previous work has centered around identifying the immunological factors which regulate allograft rejection or survival. In general, the results have demonstrated that nerve allografts are antigenic and will be rejected unless the graft recipients receive immunosuppressive treatment (e.g., cyclosporine). Because all tissue components of nerve (Schwann cells, blood vessels and perineurial sheath) survive in immunosuppressed hosts, it was not surprising to find that host axons regenerated through allografts in numbers comparable to that seen in non-antigenic, non-immunosuppressed autografts or isografts. Unfortunately, immunosuppressive treatment must be administered continually, since after stopping therapy, allografts are rejected and the host axons in them degenerate.

Since the chronic use of immunosuppressive agents entails risks, their use may not be justified for graft repair of non-life-threatening nervous tissue injury. Accordingly, studies are underway to attempt to eliminate allograft antigenicity or to devise methods to induce allograft tolerance.

One aspect of nerve graft function that has not been thoroughly investigated concerns its barrier properties. Normally, nerve fibers in the endoneurium are protected from agents (infections, proteins) around the nerve by the perineurial sheath while the movement of intravascular substances is regulated by endoneurial blood vessels. Anatomically, the permeability barriers of nerve, referred to as the perineurial nerve barrier (PNB) and blood nerve barrier (BNB) arise because of the development of tight junctions between the perineurial sheath cells of the PNB and the endothelial cells of the endoneurial blood vessels of the BNB. Last year, the PNB and BNB were studied with the barrier tracer protein, horseradish peroxidase (HRP), in rats that received nerve allografts and cyclosporine immunosuppression. The results of that study revealed that the nerve barriers were permeable to HRP during the period of Wallerian degeneration in the grafts but were normal (impermeable) in long-term recovered grafts (i.e., grafts which contained regenerated and remyelinated host axons). Recently, dead nerve grafts or artificial tubes (e.g., silicone) have been shown to accommodate axonal growth through them. However, unlike living nerve grafts where all tissue components survive, these reformed nerve segments must regenerate a new perineurium and blood vessel endothelium if barrier function is to be present. Accordingly, investigations have been carried out to determine the status of the PNB and BNB in tissue formed in rejected nerve allografts (i.e., dead nerve grafts) or in silicone tubes. In addition, the barriers were studied in cryopreserved grafts (i.e., grafts treated with protective agents that keep the tissue alive despite prior freezing, storage and thawing). Experiments were also conducted to see if cryopreservation altered allograft antigenicity. The results of these studies indicated that the nerve barriers were not restored in dead nerves or tubes but that they were intact in the cryopreserved grafts. Consequently, the clinical use of dead nerve grafts or artificial tubes seems ill advised even though they permit axonal growth and do not require immunosuppression. Cryopreserving and storing nerve allografts for two hours to five weeks did not alter their antigenicity since they were rejected by normal rats and survived in immunosuppressed ones. It appears that while nerve allograft banking is possible, these grafts nevertheless will require immunosuppression for their survival.

The research in the Section on Neuropharmacology continues to focus on the role of catecholaminergic neurons in the central and peripheral sympathetic nervous systems in regulation of normal functions, in responses to stress, and in neuronal degenerative disorders. The major catecholamines - dopamine, norepinephrine, and epinephrine - are neurotransmitters which have important roles in regulation of movement control, blood pressure, body temperature, neuroendocrine secretion, mental functions, etc. The projects in the Section are divided in those concerned primarily with catecholamine metabolism and use of assay for the amines and their metabolites to evaluate catecholaminergic function in health and disease and those which are based on examining the mechanisms and functional consequences of MPTP toxicity, which had been demonstrated to produce a primate model of Parkinson's disease useful for study of motor dysfunction and both pharmacological and tissue implant therapies.

The study of catecholamines, their precursors, and their metabolites in body fluids or after release from tissues *in vitro* has been enhanced by the development of sensitive specific methods using high performance liquid chromatography and electrochemical detection (HPLC-ED). Using a technique he developed, Eisenhofer showed that in humans monoamine oxidase inhibition not only decreases plasma levels of 3,4-dihydroxyphenylglycol (DHPG), the primary deaminated product of norepinephrine, and of 3,4-dihydroxyphenylacetic acid (DOPAC), the deaminated product of dopamine, but also depresses significantly the levels of the catecholamine precursor 3,4-dihydroxyphenylalanine (DOPA). This suggests that formation of DOPA from tyrosine was decreased, presumably as a result of feedback inhibition of tyrosine hydroxylase by elevated cytoplasmic levels of catecholamines. The effects of reserpine (to block intraneuronal storage of norepinephrine) and of desipramine (to prevent norepinephrine reuptake after release) on DHPG formation has been examined in the isolated vas deferens. The results of these studies showed that 70-80% of NE recaptured from that released during stimulation is sequestered in storage vesicles for re-release. Comparing specific activities of ^3H -NE in the tissue with that released during stimulation, it was calculated that NE was released from a small pool of NE (<1% of endogenous) which had captured a significant fraction (about 30%) of exogenous ^3H -NE. Tyramine-induced release of NE was found to produce greater proportion of DHPG, consistent with its effects in displacing NE from the storage sites into the cytoplasm; the release is non-

exocytotic. In humans, from the differences in DHPG formation from endogenous and intravenously administered NE, it was concluded that DHPG was formed mainly intraneuronally, either from NE leaking from the vesicle or from NE taken up after its release, and that increments in NE concentrations at the neuroeffector junction might be estimated from simultaneous measurement of DHPG and NE during NE infusion and during NE release.

It was shown that because of regional changes in sympathetic activity and blood flow during exposure of humans to a psychological stress (mental challenge), changes in plasma levels of NE in peripheral nervous or even arterial blood were not as accurate as measurements of total body or regional pharmacokinetic analysis of NE release reflecting changes in NE release.

Studies of urinary excretion of metabolites of dopamine and norepinephrine in patients with orthostatic hypotension suggested that homovanillic acid (HVA) is derived from dopamine formed in sympathetic neurones (as a precursor for NE) as well as from that utilized in dopaminergic neurones. It was predicted that with appropriate pharmacological inhibition of formation of both dopamine and NE metabolites in peripheral tissues, (e.g., by use of debrisoquin), it would be possible to estimate the rate of HVA formation in brain. This prediction was tested and confirmed in monkeys treated with MPTP (see below).

MPTP was discovered to cause selective damage to terminals of dopaminergic neurones in the nigrostriatal pathway of primates and to cause death of these cells. It was found to have similar, but less specific effects when given in high doses to mice; but in this species the toxic effects appear to be largely reversible since most neurones were not destroyed. Destruction by MPTP of dopamine neurones in mice was shown to be potentiated markedly by diethyldithiocarbamate (an inhibitor of superoxide dismutase and aldehyde dehydrogenase) and more recently by alcohol or acetaldehyde. Since potency of MPTP in producing parkinsonism appears to vary widely among drug abusers exposed to the toxin, concurrent use of alcohol might be a toxicity factor.

The primate model of Parkinson's disease caused by MPTP has been refined by unilateral MPTP administration, via the internal carotid artery, to produce hemiparkinsonian animals. Bankiewicz et al. have been using hemiparkinsonian animals to examine functional recovery after administration of drugs or after tissue implantation. Not only do these animals require less care since they develop symptoms on only one side, they can feed themselves and are able to sustain good health, but they turn to the lesioned side during locomotor activity. Reversal of the direction of turning after dopamine agonist, e.g., apomorphine, (due to the demonstrated development of excess number of dopamine receptors in the MPTP treated striatum), provides a useful index of severity of the hemiparkinsonism. Reversal of hemiparkinsonism by use of adrenal medulla tissue implants is transient (peak at 3 months) with subsequent regression (by 6 months) to a state slightly improved when compared with other tissue (adrenal cortex, fat) implants. Fetal monkey mesencephalic implants produce more permanent improvement. Histological examination of the brain indicates that functional improvement may be related to stimulation of ingrowth of sprouts from intact dopaminergic neurones rather than of re-nervation from the implant. This might explain bilateral recovery of parkinsonian symptoms claimed to have been achieved with unilateral autologous adrenal implants in humans.

Acute and long-term (3-6 months) alterations in local cerebral glucose utilization (LCGU) in MPTP-treated monkeys (both full and hemiparkinsonian) have been examined with and without DOPA treatment. Acute effects differ from the effects seen after stabilization of the parkinsonian syndrome. There is involvement of the substantia nigra, caudate putamen, and the regions of neuronal outflow from the basal ganglia. Motor and visual areas of the cortex are involved. In hemiparkinsonian animals, the appearance of ocular dominance columns in the striate cortex indicate prechiasmatic (presumably retinal) involvement. After DOPA administration to parkinsonian monkeys, levels of LCGU are markedly increased (to above normal levels) in those areas showing depressed LCGU. This effect is not seen in normal animals and is presumed to represent hyper-reactivity based on super-sensitive dopamine (and perhaps other) receptors.

As indicated above, urinary excretion (and plasma content) of HVA is derived from both noradrenergic and dopaminergic neurones. There is therefore a highly significant covariance of noradrenaline metabolites (MHPG and VMA) with HVA. Pharmacological depression of catecholamine formation in noradrenergic neurones of the peripheral sympathetic nervous system is attended by decreases in both dopamine and NE metabolites. The decline in excretion (or plasma levels) of these metabolites is not proportionally equal, since HVA production by brain dopaminergic neurones is unaffected. Extrapolation of the production rate of HVA to complete elimination of NE metabolites should provide an estimate of HVA production by brain. Using debrisoquin, we have shown that HVA excretion attributable to brain dopamine is diminished by about 75% in MPTP treated monkeys, consistent with extensive destruction of the nigrostriatal dopaminergic neurones which contain about 80% of brain dopamine. This data provides the basis for a method to measure HVA production in human brain from its urinary excretion (or plasma levels).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZOINS 02254-11 LNGR
PERIOD COVERED October 1, 1986 through September 30, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Repair of Injured Nervous Tissue With Foreign Grafts		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I. : A.A. Zalewski, M.D., Section Head, LNGR, DIR, NINCDS Others: Y. Kadota, M.D., Visiting Associate, LNGR, DIR, NINCDS N.A. Azzam, Ph.D., Guest Researcher, LNGR, DIR, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuronal Growth and Regeneration, DIR, NINCDS		
SECTION Section on Neuronal Development and Regeneration		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 3.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The passage of large molecules into the endoneurial environment of large <u>peripheral nerves</u> is regulated by two distinct barriers, the <u>perineurial nerve barrier</u> (PNB) and the <u>blood nerve barrier</u> (BNB). The PNB protects the endoneurium from entrance of agents that might be present around the nerve, whereas the BNB regulates the movement of intravascular agents out of endoneurial blood vessels. Anatomically, the PNB and BNB arise respectively because of the formation of tight (impermeable) junctions between the <u>perineurial sheath cells</u> and the <u>endothelial cells</u> of endoneurial blood vessels. In the present experiments, we have examined in rats at the light and electron microscopical level whether a PNB and BNB form in a segment of nerve formed in a rejected nerve or in a silicone tube. In addition, the barriers were studied in cryopreserved (i.e., living not frozen killed) <u>nerve isografts</u>. We also determined whether cryopreservation (in dimethyl sulfoxide and formamide) altered <u>nerve allograft antigenicity</u> in rats. Normally, the barrier tracer protein horseradish peroxidase (HRP, an enzyme of 40,000 M.W.) is kept out of the endoneurium by the tight junctions of the PNB and BNB. However, reaction product for HRP activity was observed in the endoneurium of the nerve segments that reformed after 4-6 months in rejected nerves or in silicone tubes, indicating that these barriers were leaky. In contrast, the barriers were intact in cryopreserved nerve. Our data indicate that barriers do not develop in rejected nerve or in silicone tubes even though these conduits permit axonal regeneration through them. <u>Cryopreserving nerve allografts</u> for 2 hours to 5 weeks did not alter their antigenicity. These grafts were rejected by non-immunosuppressed rats but survived in hosts treated with the immunosuppressive agent <u>cyclosporine</u>. Our results indicate the feasibility of banking nerve allografts, but their survival, after grafting, still requires immunosuppression. *[Transferred to LNGR 10/86 from LNC]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02716-02 LNGR
PERIOD COVERED October 1, 1986 through September 30, 1987 **		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) MPTP Toxicity and Animal Models of Parkinson's Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Irwin J. Kopin, M.D., Chief, Neuropharmacology Section, LNGR; Director, DIR, NINCDS Others: Krzysztof S. Bankiewicz, M.D., Visiting Fellow, SNB, NINCDS Virginia Weise, Neuropharmacology Section, LNGR, NINCDS Urmi Patel, Ph.D., Senior Staff Fellow, Neuropharmacology Section, LNGR, NINCDS Sergio Schinelli, M.D., Ph.D., Visiting Fellow, Neuropharmacology Section, LNGR, NINCDS Judith Harvey-White, B.S., Technician, Neuropharmacology Section, LNGR, NINCDS *		
COOPERATING UNITS (if any) Surgical Neurology Branch, NINCDS Laboratory of Clinical Science, NIMH		
LAB/BRANCH Laboratory of Neuronal Growth and Regeneration		
SECTION Neuropharmacology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.2	PROFESSIONAL: 2.2	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which causes selective destruction of dopaminergic neurones of the nigrostriatal pathway in monkeys, but is less specific in mice requiring higher doses and affecting other areas as well. A hemiparkinsonian model has been developed by infusion of MPTP into the internal carotid artery of monkeys. The behavioral effects (turning in the direction of the lesioned side) are used to evaluate agonists (which reverse the turning) and procedures which regenerate or replace by implantation dopamine-producing cells. Dopaminergic neurones and receptor changes have been demonstrated using biochemical techniques for measurement of dopamine, norepinephrine, and serotonin and their metabolites as well as binding of radiolabelled ligands to receptors and immunohistochemistry of tyrosine hydroxylase. These confirm unilateral destruction of dopamine innervation of the caudate-putamen in hemiparkinsonian monkeys and increased D₂ receptors in this area in these animals. PET scanning with ¹⁸F-DOPA has demonstrated the deficiency in dopamine formation and storage in the striatum of MPTP treated animals and the restoration of dopamine in transplants of fetal mesencephalic tissue. Tyrosine hydroxylase immunohistochemical studies of the results of tissue implants suggest stimulation of sprouting of surviving dopaminergic neurones rather than fiber ingrowth from the implant as the cause of functional recovery. There are also functional restorations of local cerebral glucose utilization (LCGU) in areas of the cortex and striatum in which LCGU is depressed by MPTP. Using LCGU to examine responses to DOPA, it was found that supersensitivity was evident in that LCGU was markedly elevated in MPTP-treated, but not in normal monkeys by L-DOPA. In hemiparkinsonian monkeys, the ocular dominance columns could be distinguished and those innervated by the MPTP treated-eye reacted to L-DOPA in a similar hyperactive manner, suggesting retinal dopamine receptor supersensitivity. * Continued: Alessandro Zuddas, M.D., Visiting Fellow, Neuropharmacology Section, LNGR, NINCDS Linda Porrino, Ph.D., Research Psychologist, NIDA ** [Transferred to LNGR 10/86 from NI]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 NS 02717-02
 LNGR

PERIOD COVERED

October 1, 1986 through September 30, 1987 **

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Evaluation of Aminergic Function***

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Irwin J. Kopin, M.D., Chief, Neuropharmacology Section, LNGR; Director, DIR, NINCDS
 Others: Graeme Eisenhofer, Ph.D., Visiting Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS
 Zofia Zukowska-Grojec, M.D., Ph.D., Guest Researcher, Neuropharmacology Section, LNGR, DIR, NINCDS
 Moshe Garty, M.D., Visiting Scientist, Neuropharmacology Section, LNGR, DIR, NINCDS
 Mark Duncan, Ph.D., Visiting Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS
 David Hovey-Sion, Ph.D., Visiting Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS
 *

COOPERATING UNITS (if any)

Hypertension-Endocrine Branch, NHLBI
 Medical Neurology Branch, NINCDS

LAB/BRANCH

Laboratory of Neuronal Growth and Regeneration

SECTION

Neuropharmacology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.2

PROFESSIONAL:

3.2

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Measurement of levels of norepinephrine (NE), its metabolites, and precursors in plasma, urine, and cerebrospinal fluid have been developed and are being applied to studies of changes of these compounds in patients with various disorders of the autonomic nervous system and in rats after administration of drugs which alter synthesis, storage, release, metabolism, or uptake of NE in sympathetic nerves. Drugs such as desipramine (or cocaine) which block uptake, enhance apparent release of norepinephrine into the circulation and diminish formation of its deaminated metabolites, DHPG and MHPG. L-DOPA is formed in the sympathetic neurones and overflows into the blood. It may be an important source of extraneuronal dopamine and act as a neurohumoral agent. Patients with orthostatic hypotension have parallel decreases in excretion of homovanillic acid (HVA), the major metabolite of dopamine, and norepinephrine metabolites (MHPG and VMA). A similar relationship holds in animals and humans treated with debrisoquin to inhibit peripheral catecholamine metabolism. Using monkeys treated with MPTP to destroy central dopaminergic neurones, we have established the validity of extrapolating the regression line relating HVA to MHPG to measure the brain HVA production. This has been done in both urine and plasma of monkeys. A means of rapid enzymatic conversion of ¹⁸F-dopa to ¹⁸F-dopamine has been developed. The ¹⁸F-dopamine is to be administered as a precursor of endogenous ¹⁸F-norepinephrine to study by PET scanning the peripheral sympathetic neurones in various disease states.

*Continued:

Anna Starosta, M.D., Visiting Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS
 Katalin Szemerédi, Ph.D., Visiting Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS
 Ronald Polinsky, M.D., Chief, Clinical Neuropharmacology Section, MNB, DIR, NINCDS
 David Goldstein, M.D., Ph.D., Medical Officer, Hypertension-Endocrine Branch, NHLBI

***[Formerly: "Biochemical Evaluation of Adrenergic Function"]

[** Transferred to LNGR 10/86 from NI]

9 LNGR/DIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02752-01
LNGR

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Synthesis and Expression of Neurotrophic Agents and Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Joan P. Schwartz, Ph.D., Research Chemist, Neuropharmacology Section, LNGR, DIR, NINCDS
 Others: Hisaharu Shinoda, M.D., Visiting Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS
 Margaret Palmatier, Ph.D., Staff Fellow, Neuropharmacology Section, LNGR, DIR, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuronal Growth and Regeneration

SECTION

Neuropharmacology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence suggests that parallel biochemical and regulatory processes occur during normal development and following various forms of CNS injury. Among the areas of particular interest are (1) the identification of CNS neurotrophic factors, and (2) the analysis of the regulation of neuropeptide gene expression during development and in response to injury. Studies are underway to identify trophic factors produced in two specific model systems, since recent evidence suggests that a family of nerve growth factors exists, each specific for certain populations of neurons. Mouse cerebellum contains a factor which is NGF-like by immunological criteria but has no biological activity on chick sensory neurons. This factor increases in the cerebellum of the pcd mutant mouse as the Purkinje cells die out and astrocytes proliferate. The mRNA for this factor appears to hybridize with mouse β -NGF cDNA and is increased in pcd cerebellum. Screening of cDNA libraries is currently in progress, in order to clone the factor. In another injury paradigm, multiple cortical lesions are made in rat brain: one week later, RNA is prepared from various brain regions to be analyzed for any change in NGF-like mRNA's relative to unlesioned brain.

At the same time, these injury models can be evaluated for changes in neuropeptide and/or neurotransmitter synthesis occurring in response to the lesions. One can derive an estimate of peptide turnover by combining measurements of the precursor mRNA, the precursor, and the peptide. Our studies have demonstrated that peptides are differentially regulated by such chronic drug treatments as reserpine, haloperidol, 6-hydroxydopamine or 5,7-dihydroxytryptamine. Work is in progress to determine the effects of CNS injury and recovery on various neuropeptides as well as such neurotransmitter synthetic enzymes as tyrosine hydroxylase and GAD.

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ANNUAL REPORT
October 1, 1986 through September 30, 1987

Biometry and Field Studies Branch

Division of Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

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Biometry and Field Studies Branch
Division of Intramural Research
National Institute of Neurological and Communicative Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Chief

The Biometry and Field Studies Branch (BFSB) supports a program in biostatistics to advance the mission of NINCDS in the areas of neurologic and communicative disorders. The Branch participates in a wide range of intramural and extramural collaborative projects, including large- and small-scale observational studies, clinical trials and laboratory studies. These collaborative studies are conducted both through direct staff research and through research and development contracts. In addition to collaborative work, the Branch has an important research component in statistical methodology.

The Branch is composed of an Office of the Chief and three Sections. The Mathematical Statistics Section, headed by Dr. James Dambrosia is the main statistical consulting unit for other branches and laboratories in the Division of Intramural Research as well as the Extramural Research Divisions. This Section is also responsible for research in statistical methodology. The Computer Applications Section is occupied mainly with the activities of the Stroke and Traumatic Coma Data Banks. The Branch Chief is Acting Chief of this Section, although Dr. Mary Foulkes is Project Director for both of the Data Banks. The Data Processing Section provides computer programming, systems analysis, and data management support to the Branch and is headed by Ms. Sylvia Edelstein. Dr. James Dambrosia has been appointed Deputy Chief of the Branch. In that capacity he assists the Branch Chief in policy decisions and assumes part of the administrative burden of running the Branch. Dr. Richard Raubertas, after a very productive three-year tenure with the Branch, has accepted a joint faculty position in the Department of Biostatistics and the Medical School at the University of Rochester. Our recruitment efforts to fill vacant positions for entry level biostatisticians have been hampered by increased competition for expertise in this area and a major differential in staff fellow salary levels for entry level biostatisticians as compared to that in other academic institutions and industry.

Many of our currently supported projects are long-term and labor intensive. These projects require an enormous amount of effort to be devoted to routine activities such as data entry, data editing and day to day monitoring of protocol compliance. This type of activity, although essential, consumes a large amount of our staff time, with the drawback that little time is left for statistical methodology and expansion of our collaborative efforts into new areas. BFSB collaboration on new large-scale projects with a substantial data management component will be carefully considered, and our participation will depend in large part, on whether routine data management operations of such studies can be contracted out, under our supervision. We have included in our

budget for FY '88, direct operations monies to fund a data management and programming contract which will provide data entry, data monitoring, computer programming and systems analysis in partial support of active and new collaborative projects. We expect that such a contract will be effective at the beginning of FY '88. By the end of FY '89, most of the data collection and editing aspects of our current large-scale projects will be completed.

I. COLLABORATION WITH THE INTRAMURAL AND EXTRAMURAL RESEARCH DIVISIONS, NINCDS

Our current program of collaborative research has developed primarily in response to requests for collaboration from intramural and extramural scientists at NINCDS. Typically, BFSB assumes responsibility for the statistical design, data management, statistical analysis, and interpretive aspects of the projects, with the Divisions providing the project initiatives, subject matter expertise, and overall leadership. In addition the Branch is engaged in research in statistics as it applies to problems encountered in clinical, laboratory, and epidemiological studies.

In collaboration with the Division of Convulsive, Developmental and Neuro-muscular Disorders, (DCDND), BFSB is the statistical coordinating center for the clinical trial of behavioral and cognitive side effects of phenobarbital used for the prevention of febrile seizure recurrence. Patient accrual has been completed, with 217 children with febrile seizures randomized to treatment and 150 seizure free controls recruited for the study. Follow-up of patients will continue until June, 1988. BFSB has been the comprehensive operations center for this study, which has required extensive monitoring of patient accrual and data quality control and several interim data analyses for the trial's monitoring committee.

A second collaborative effort with the DCDND is a population-based study of the prognostic value of the EEG for subsequent seizure activity in children who experienced a febrile seizure. The cooperating medical center is the Pediatric Clinic in Skopje, Yugoslavia. The recruitment of new cases ended in December, 1984, and periodic follow-up (including repeat EEGs and neurologic and physical examinations) will continue through December, 1987. The study includes 400 children with a normal or non-specific abnormal EEG following a first febrile seizure, as well as about 300 children with a specific abnormal EEG following a seizure. The major outcomes of the study are recurrent febrile and afebrile seizures and their relationship to the initial EEG, subsequent EEG changes, and the influence of other medical and demographic factors.

BFSB is participating with the Division of Communicative Disorders on a study of factors associated with the acquisition of reading and writing skills by the deaf. Information on audiologic, educational and family background, and on language skills will be examined to determine which, if any, of these variables are associated with reading and writing skills in deaf adolescents. A pilot study has been completed and the main data collection phase is now in progress. Some research will be required to develop appropriate statistical techniques for measuring the association of background and language factors with reading and writing skills.

Seven therapy intervention studies for the treatment of acute stroke are in various stages of completion. These studies are conducted under the Cerebrovascular Clinical Research Master Agreement of the Division of Stroke and Trauma. The primary role of BFSB in these studies is that of data management.

A dose escalation study of naloxone with acute cerebral ischemia has been completed and maximum tolerated dose, and adverse effects were established. A Phase II study of naloxone was also completed and analysis files have been constructed. Data collection for a pilot study of the benefits of hypervolemic hemodilution (dextran-40) for the treatment of stroke-in-evolution, has also been completed. A dose-escalation Phase II study of nicardipine, a calcium channel blocker, for the prevention of vasospasm following subarachnoid hemorrhage, and Phase II studies of heparinoid, nicardipine, and rt-PA for the treatment of acute cerebral ischemia are ongoing. Patient accrual and data collection for these studies will be completed by the end of FY '88.

The BFSB continues to collaborate with many Branches and Laboratories in the Division of Intramural Research (DIR). The Survey of Major Neurological Disorders in Copiah County, a joint project with the Neuroepidemiology Branch, has been completed and all primary papers reporting the results have been published. The methodology developed for this project, which provides an effective model for other studies attempting to obtain prevalence rates of neurologic disorders in geographically defined populations, has been described in the published reports.

BFSB is working with the Medical Neurology Branch on a clinical trial of felbamate, a new drug for the treatment of intractable partial seizures. The study uses a randomized, double-blind, three-period-crossover design, allowing unbiased estimation of treatment effect even in the presence of period-to-period carry-over effect. Approximately half of the required number of patients have completed the study.

Other collaborative studies in DIR include: statistical aspects of the epidemiology of Creutzfeld-Jakob disease with emphasis on clustering (LCNSS); comparison of the relationship between the number of myelin lamellae and axon size in normal and "shiverer" strains of mice (LENP); a pilot study of von Recklinghausen's neurofibromatosis (NE); analysis of time to stroke using time dependent covariates in a proportional hazards regression model (NE); a clinical trial of ganglioside therapy for diabetic neuropathy (MN); a study of the potential association of toxoplasmosis during pregnancy with morbidity in the child (ID); analysis and comparison of the amplitude of blink responses evoked by mechanical or electrical stimuli (MN); a study of IL-2 and LAC cell therapy for brain tumor in a rat model (SN); an evaluation of stereotactic implantation of dispersed cells into the brain (SN); logistic discrimination of epilepsy patients with complex partial seizures into tractable and intractable groups (NE and MN); and a case-control study to identify predictors of febrile seizures based on data from six Chinese cities (NE).

The major analyses of data from the Collaborative Perinatal Project (NCP) in collaboration with DCDND, were completed in FY '86. Work on the maternal infection studies from this data base was completed in FY '87. A study of the relationship of toxoplasmosis during pregnancy with childhood outcome has shown increases in the risk of deafness, microcephaly and low IQ among children born

to women with high maternal antibody to toxoplasmosis. A case control study assessing the potential impact of the presence of 11 infections during the second and third trimester of pregnancy did not demonstrate a clear association between infection and childhood outcome. Both papers have been submitted for publication. A study investigating the association of migraine with other diseases has been completed and will be submitted for publication, and another assessing the familial relationship of migraine in mothers and morbidity in their children is in progress.

II. CLINICAL DATA BANKS

BFSB continues its responsibility for the funding, management and operation of the Stroke and Traumatic Coma Data Banks. Each data bank is a collaborative effort between BFSB, which is the statistical coordinating center, and four hospital centers. The data banks involve the collection of prospective, observational, clinical and laboratory data at the multiple clinical centers using a common set of data forms. These data banks provide a resource for addressing research questions on the characteristics, clinical course, and outcome of hospitalized stroke and traumatic head injury patients.

Data collection, processing and editing involve the coordinated efforts of BFSB, a contractor for systems analysis and computer programming support (Rush Associates Inc.), and the clinical data bank centers. BFSB has designed and implemented a data base system at NIH (DCRT) and a patient tracking system to monitor patient accrual and completed follow-up visits. The Rush Associates workscope has been expanded from design, maintenance, and telecommunications aspects of the "front-end" micro-computer system to include transfer of data from the micro-computer to DCRT, and programming support for the building, updating, and editing of the databases at DCRT. DCRT is the data repository for each of the data banks.

For the duration of both data banks, BFSB will continue to emphasize monitoring of data collection to enhance consistency of procedures at the collaborating centers, to require prompt entry of collected data, and to increase the rate of successful follow-up. Resources will be allocated to ensure satisfactory databases when these studies are completed. Any major efforts at analysis, publication of preliminary results or any other uses of resources which put achievement of this objective at risk will be avoided.

Stroke Data Bank

Data collection for the main phase of the Stroke Data Bank began in FY '83. By the end of new patient accrual in June, 1986, 1,805 patients had been entered. All acute care data were entered by the centers, edited and corrected by BFSB and a final acute care data file was created in September, 1986. Primary analysis of acute care data began at that time. Collection of follow-up data continued until March, 1987, and the final edited data base was completed in May, 1987. A priority for analysis and publication has been established to

focus on the areas of primary interest. A policy for publication and a Publication Committee were established to prioritize, review and critique all potential publications.

Examples of research studies being addressed include: the identification of patients at risk for evolving ischemic stroke; the investigation of racial and sexual differences in stroke type, site and vascular territory; and the examination of stroke severity to determine whether motor weakness and/or sensory loss can be predicted by the location or size of the CT scan abnormality in infarcts. A logistic regression model based on the Pilot Stroke Data Bank patient records has been developed to identify early during hospitalization a group of patients unlikely to survive for one month following their intra-cerebral hemorrhage. This model is now being evaluated for its predictive ability in the main phase of the Stroke Data Bank. In addition, the characteristics of the diagnostic subtypes of lacunar and infarcts of undetermined cause have been examined in detail.

Traumatic Coma Data Bank

Data collection for the main phase Traumatic Coma Data Bank began in FY '84. By the end of September, 1987, approximately 1000 severely head-injured patients will have been enrolled in this project.

The first major analyses will begin after accrual of patients has been completed in September, 1987, and the acute care data has been entered and edited. The focus of the Branch this year has been monitoring of the project with regard to completeness and quality of data collection. In addition, emphasis has been placed on identification of those primary questions for which the data are the most complete and of highest quality. Initial assessment of analysis approaches will be undertaken in these areas. Continued emphasis has been placed on consistent, unbiased, and complete follow-up of surviving coma patients to permit the detailed evaluation of outcome, especially in the area of neuropsychological and neurobehavioral function and quality of life assessment following severe head injury.

III. METHODOLOGICAL RESEARCH IN STATISTICS

BFSB statisticians continue to develop new statistical methodology and derive innovative modifications of statistical techniques to meet the needs of the Institute for the design of experiments and field studies, analysis of data, and statistical modeling of biological processes and phenomena. Most of the statistical problems addressed arise from collaborative studies with the Intramural and the Extramural Divisions. In general, there are two objectives associated with these various statistical activities of BFSB. The primary objective is the development and improvement of statistical methodology to meet the needs of the Institute. The secondary objective is to make contributions to the development of statistical methodology which may be more generally useful in neurologic and other medical research.

A partial listing of areas in which BFSB staff is developing new statistical applications to neurologic problems includes: modified tests for space-time clustering of rare disease applied to a population in a defined geographic area; use of incomplete observations in statistical models derived by stepwise variable selection procedures; methods for adjustment of the effect of concomitant variables in categorical data analysis; sampling strategies for rare neurologic disorders; the application of proportional hazards regression with time dependent risk factors to the analysis of stroke etiology; determination of the order of categories in attribute data; and methods of inference on frequency of events in follow-up data.

Theoretical statistical work has included: the effect of misclassification of exposure variables on case-control studies; the non-null distribution of statistics that measure spatial clustering; new hypothesis testing procedures in the presence of inequality constraints; nonparametric methods for quadratic alternatives; the effect of informative censoring on survival analysis; examination of the biases introduced by left-truncation of time-dependent covariates in the analysis of proportional hazards; pooled adjacent violators; comparison of common parametric and nonparametric methods for survival analysis in the presence of mismodeling; proportional hazard model estimators for transitions in discrete state semi-Markov processes; sample size and power calculations for survival studies with other than the usual accrual, dropout and compliance assumptions; and stochastic curtailing for comparison of slopes in longitudinal studies.

IV. OUTSIDE ACTIVITIES

Several of our staff have been active on important national and international review committees, and have participated in major meetings or received other peer recognition this year. Dr. Ellenberg has been elected President of the International Biometric Society. He was also honored this year with the United States Public Health Service Superior Service Award. He was invited to lecture at the University of California Biostatistics Department. Dr. Dambrosia has been selected as the Biometrics Program Chairman for the 1988 annual meeting of the American Statistical Association. Dr. Foulkes is a member of the monitoring committee for the VA Cooperative Study of Carbamazepine Versus Valproic Acid for Treatment of Partial Seizures. Dr. Anderson presented two plenary lectures at the Pan American Congress of Neuroepidemiology in Santiago, Chile on the design and quality control of epidemiological surveys. Dr. Lee was invited to the First International Conference on Statistical Computing in Izmir, Turkey where he presented a paper on nonparametric statistical methods in quadratic alternatives. Dr. Lee presented two invited papers, one on nonparametric methods in quadratic regression and another on statistics in bioassays and in-vitro mutation assays, at the Tenth Korean Symposium on Science and Technology in Seoul, Korea.

In summary, BFSB is involved in a strong program of collaborative research. Our collaboration extends throughout the Institute on projects with both Intramural and Extramural scientists, and also involves collaboration with scientists outside of NINCDS. The scope of our research activity ranges from small, one-on-one collaboration with intramural scientists, to the conduct of large-scale, multicenter clinical studies. BFSB also makes an important and continuing contribution to statistical methodology applicable to neurological research.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, DIR, NINCDs
Fiscal Year 1987

1. UNIV. OF MARYLAND (N01-NS-2-2302)
2. NEUROLOGICAL INSTITUTE - COLUMBIA UNIV. (N01-NS-5-2384)
3. BOSTON UNIV. (N01-NS-2-2398)
4. MICHAEL REESE HOSPITAL & MEDICAL CENTER (N01-NS-2-2399)

Title: Full Phase Stroke Data Bank

Date Contracts Initiated: July 1, 1982

Contractors' Principal Investigators:

1. Dr. Thomas Price
2. Dr. Jay Mohr
3. Dr. Philip Wolf
4. Dr. Daniel Hier

Current Annual Levels FY '87*:

1. \$0
2. \$0
3. \$0
4. \$0

* Final incremental funding for these projects appropriated in FY '86

Objectives: The primary objective of this project is to implement the full phase of the Stroke Data Bank (SDB) study, which will collect observational acute and long-term longitudinal data on stroke patients. The data bank will provide a resource for clinical research studies of patients with stroke. This is a collaborative project which involves four clinical centers and BFSB. The clinical centers are responsible for the collection of data and collaboration on the development of and analysis of research questions. The BFSB, is the statistical coordinating center, responsible for maintenance of the centralized data base management system for transmission, for storage and retrieval of data; for monitoring of data acquisition and data quality; and for collaboration with the clinical investigators on the analysis of data concerning the primary research questions of the data banks.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the first year of this project, outlined research objectives, and developed forms and data collection procedures. The focus this fiscal year has been on the completion of patient accrual, the improvement of follow-up rates, the elimination of data entry backlog, the creation of edited data files for analysis, the development and implementation of a publications policy and a publications committee to prioritize and to review and critique all potential publications, and the creation of analysis teams for the primary research questions. In addition, a monitoring committee was convened by the Branch to provide continuing critical review of the Stroke Data Bank to both the Branch Chief and the DIR Director.

Major Findings: A mathematical model for identifying patients at greatest risk for progression of stroke has been developed based on the Pilot Stroke Data Bank, validated and revised based on the Main Phase SDB. The characteristics of the diagnostic subtypes of lacunar and infarcts of undetermined cause have been examined in detail. Mathematical models for both infarcts and intracerebral hemorrhages have been developed to identify patients unlikely to survive for one month following their stroke. Patient and lesion characteristics detected on CT scan have been identified that are associated with silent and unreported old strokes.

Significance to the NINCDS Program and Biomedical Research: Acute care and longitudinal data on stroke patients have been collected at four centers, using uniform definitions and procedures. This information now provides a large body of data for clinical research on the factors influencing survival and quality of life following a stroke.

Proposed Course of the Project: This is the beginning of the fifth year of a five-year project. Work in the first year included determination of research questions to be investigated and design of forms to collect the data. Data collection continued July, 1983, through May, 1987, and information on 1805 patients has been collected.

Publications:

Gross CR, Shinar D, Mohr JP, Hier DB, Caplan LR, Price TR, Wolf PA, Kase CS, Fishman IG, Calingo S, Kunitz SC. Inter-observer Agreement in Clinical Studies: Application to the Diagnosis of Stroke Type. Arch Neurol 1986;43(9):893-898.

Bronstein K, Murray P, Licata-Gehr E, Banko MA, Kelly-Hayes M, Fast S, Kunitz S. Implications for Nursing Research. J Neurosci Nurs 1986;18(3):132-134.

Mohr JP. Stroke Data Banks. Stroke 1986;17:171-172.

Wolf CL, Guthrie B. Computerized Clinical Data Bank Network. Proceedings of the SAS Users Group International 1986;11:324-328.

Shinar D, Gross CR, Hier DB, Caplan LR, Mohr JP, Price TR, Wolf PA, Kase CS, Fishman IG, Barwick JA, Kunitz SC. Interobserver Reliability in Interpretation of Computed Tomographic Scans of Stroke Patients. Arch Neurol 1987;44:149-155.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, DIR, NINCDS
Fiscal Year 1987

- | | |
|---------------------------------------|-----------------|
| 1. <u>UNIV. OF TEXAS-GALVESTON</u> | (N01-NS-3-2339) |
| 2. <u>UNIV. OF CAL. IN SAN DIEGO</u> | (N01-NS-3-2340) |
| 3. <u>MEDICAL COLLEGE OF VIRGINIA</u> | (N01-NS-3-2341) |
| 4. <u>UNIV. OF VIRGINIA</u> | (N01-NS-3-2342) |

Title: Full Phase Traumatic Coma Data Bank

Date Contracts Initiated:

1.	April 15, 1983
2.	April 15, 1983
3.	June 1, 1983
4.	July 1, 1983

Contractors' Principal Investigators:

1.	Dr. Howard Eisenberg
2.	Dr. Lawrence Marshall
3.	Dr. Harry Young
4.	Dr. John Jane

Current Annual Level FY '87:

1.	\$294,155
2.	\$291,913
3.	\$245,710
4.	\$239,218

Objectives: The primary objective of this project is to implement the full phase of the Traumatic Coma Data Bank study, which will collect observational acute and long-term longitudinal data on severely head injured patients. The data bank will provide a resource for clinical research studies of patients with head injury. This is a collaborative project which involves four clinical centers and BFSB. The clinical centers are responsible for the collection of data and collaboration on the development of and analysis of research questions. The BFSB is the statistical coordinating center, responsible for maintenance of the centralized data base management system for transmission, storage and retrieval of data; for monitoring of data acquisition and data quality; and for statistical collaboration or oversight of the statistical analysis for the primary research questions of the data bank.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the initial year of this project, outlined the research objectives, and developed forms and data collection procedures. Data collection began in January, 1984. During this fiscal year, the focus has been on the improvement of follow-up rates, the assessment of center differences in data collection procedures and corrections where necessary, the general

(NO1-NS-3-2339)

(NO1-NS-3-2340)

(NO1-NS-3-2341)

(NO1-NS-3-2342)

improvement of data entry procedures and the elimination of data entry backlog. A policy for publications and a Publications Committee were established to prioritize, review and critique all potential publications. In addition, a monitoring committee was convened by the Branch Chief to provide continuing critical review of the Traumatic Coma Data Bank to both the Branch Chief and DIR Director.

Major Findings: Primary end results will be available following completion of data collection.

Significance to the NINCDS Program and Biomedical Research: Acute care and longitudinal data on head-injured victims will be collected at four centers, using uniform definitions and procedures. This information will provide a large body of data for clinical research on the factors influencing survival and quality of life following severe head injury. The number of therapies and monitoring devices commonly utilized during the acute phase of managing traumatic coma necessitates a highly organized data handling capacity, and the data bank will serve as an efficient mechanism for collecting, storing and retrieving this information as well as follow-up data.

Proposed Course of the Project: This is the fourth year of a five-year project. Data collection is continuing and as of May, 1987, over 800 patients have been entered into the project.

Publications:

Marshall SB, Cayard C, Foulkes MA, Hults K, Gauttill T, Charlebois DB, Tisdale NA, Turner H. The Traumatic Coma Data Bank: A Nursing Perspective. J Neurosci Nurs (In press)

Hinkle JL, Turner H. Three Collaborative Research Projects Involving Neuroscience Nurses. J Neurosci Nurs, 1986;18(6):354-356.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, DIR, NINCDS
Fiscal Year 1987

RUSH & ASSOCIATES, INC., Fairfax, Virginia (N01-NS-6-2305)

Title: Collection and Maintenance of Data for the Stroke Data Bank
and Traumatic Coma Data Bank Projects

Date Contract Initiated: September 1, 1986

Contractor's Project Director: Robert L. Rush

Current Annual Level FY '87: \$163,158

Objectives: To provide and maintain front-end microprocessor support for the Stroke and Traumatic Coma Data Bank projects (N01-NS-2-2302, 2398-9, N01-NS-5-2384, N01-NS-3-2339-42), with facilities for interactive data entry, updating, editing and transmissions to the NIH DCRT main-frame computer. As well, to provide data maintenance, retrieval and management of the stored data. To maintain and refine software for data monitoring procedures.

Major Findings: This contract is expected to provide substantial support for data entry, editing, storage and retrieval for both the Stroke and Traumatic Coma Data Bank projects.

Significance to the NINCDS Program and Biomedical Research: The ability to enter and check data on-site via front-end software is an integral part of the Stroke and Traumatic Coma Data Bank projects. This contract will also provide the support for data management, storage and retrieval, necessary to fulfill the research objectives of both projects.

Proposed Course of the Project: This contract will run from September 1, 1986 through December 1, 1988.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02652-03 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Intramural Statistical Collaboration and Consultation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: James M. Dambrosia, Ph.D. Chief, Mathematical Statistics Section BFSB, DIR, NINCDS Others: Dallas Anderson, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINCDS Sherrie E. Emoto, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS Mary A. Foulkes, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS Young Jack Lee, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS Richard Raubertas, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 3.2	PROFESSIONAL 3.0	OTHER 0.2
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) This project is designed to provide statistical collaboration and consultation for Laboratories and Branches within the Division of Intramural Research (DIR). Particular consideration is given to <u>statistical planning and design of experiments, statistical analysis of data, and statistical inference</u> . Our collaboration has involved eight Laboratories/Branches, and the scope of the studies has ranged from the coordination and statistical management of a complex clinical trial to consultation on the appropriateness of the statistical analysis used for small laboratory experiments. Examples of studies with DIR include a randomized clinical trial of felbamate, a new drug for the treatment of intractable partial seizures (MN); statistical aspects of the epidemiology of Creutzfeldt-Jakob disease with emphasis on case clustering (LCNSS); examination of the relationship between the number of myelin lamellae and axon size in normal and 'shiverer' strains of mice (LENP); development of proportional hazards models for time to stroke with time dependent covariates (NE); a clinical trial of ganglioside therapy for diabetic neuropathy (MN); a pilot study of von Recklinghausen's neurofibromatosis (NE); comparison of mechanically and electrically evoked blink reflexes (CNP); logistic discriminant methods for classifying patients with complex partial seizures (NE and MN); IL2 treatment of brain tumors in animal models (SN); identification of risk factors for febrile seizures with a population based case-control study of six cities in China (NE); and stereotactic implantation of dispersed cells into the rat brain with focus on placement and survival (SN).		
12 - BFSB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02490-07 BFSB																												
PERIOD COVERED October 1, 1986 through September 30, 1987																														
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Research in Statistics																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">James M. Dambrosia, Ph.D.</td> <td style="width: 20%;">Chief, Mathematical Statistics Section</td> <td style="width: 20%;">BFSB, DIR, NINCDS</td> </tr> <tr> <td>Others:</td> <td>Dallas W. Anderson, Ph.D.</td> <td>Mathematical Statistician</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Jonas H. Ellenberg, Ph.D.</td> <td>Chief</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Sherrie E. Emoto, Ph.D.</td> <td>Mathematical Statistician</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Mary A. Foulkes, Ph.D.</td> <td>Mathematical Statistician</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Young Jack Lee, Ph.D.</td> <td>Mathematical Statistician</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Richard F. Raubertas, Ph.D.</td> <td>Mathematical Statistician</td> <td>BFSB, DIR, NINCDS</td> </tr> </table>			PI:	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BFSB, DIR, NINCDS	Others:	Dallas W. Anderson, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS		Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS		Sherrie E. Emoto, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS		Mary A. Foulkes, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS		Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS		Richard F. Raubertas, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS
PI:	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BFSB, DIR, NINCDS																											
Others:	Dallas W. Anderson, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS																											
	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS																											
	Sherrie E. Emoto, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS																											
	Mary A. Foulkes, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS																											
	Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS																											
	Richard F. Raubertas, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS																											
COOPERATING UNITS (if any)																														
LAB/BRANCH Biometry and Field Studies Branch																														
SECTION Mathematical Statistics Section																														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																														
TOTAL MAN-YEARS 2.2	PROFESSIONAL 2.1	OTHER 0.1																												
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																														
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) This project addresses <u>statistical problems</u> generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics. Papers have been submitted or published in FY' 87 on the following statistical subjects: pooled adjacent violators; general test of trend for count data; hypothesis tests involving linear inequality constraints; sampling strategies for rare diseases; the development of robust selection procedures based on vector ranks; the evaluation of sample size and power for analysis of survival with allowance for nonuniform patient entry, losses to follow-up, noncompliance and stratification; stochastic curtailing for comparison of slopes in longitudinal studies; and selection criteria for use of the Kaplan-Meier or MLE for life table analysis. Other work in progress includes: statistics for the evaluation of space-time clustering of disease; adjustments for covariates in the analysis of categorical data; the influence of missing data on statistical models determined by variable selection procedures; the effects of misclassification of exposure variables on case-control studies; the use of area surveys in epidemiological research; the impact of risk and incidence of risk on selection of variables in multiple regression; the development of methods to improve coverage in surveys; the use of nonparametric tests for umbrella type alternative hypotheses; the impact of informative censoring on survival analysis with time dependent covariates; the development of methods of survival analysis for dependent competing risks; derivation of inferential methods for determining order of categorical data; comparison of common parametric and nonparametric survival analysis in the presence of missmodeling.																														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02444-08 BFSB																								
PERIOD COVERED October 1, 1986 through September 30, 1987																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Statistical Coordinating Center for the Phenobarbital Clinical Study*																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Young Jack Lee, Ph.D.</td> <td style="width: 40%;">Mathematical Statistician</td> <td style="width: 20%;">BFSB, DIR, NINCDS</td> </tr> <tr> <td>Others: Sylvia Edelstein</td> <td>Chief, Data Processing Sec.</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Jonas H. Ellenberg, Ph.D.</td> <td>Chief</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Deborah G. Hirtz, M.D.</td> <td>Pediatric Neurologist</td> <td>DNB, DCDND, NINCDS</td> </tr> <tr> <td>Dolores Jones</td> <td>Computer Assistant</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Mary F. Livingston</td> <td>Clinical Data Assistant</td> <td>DNB, DCDND, NINCDS</td> </tr> <tr> <td>Karin B. Nelson, M.D.</td> <td>Medical Officer</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Jack Panossian</td> <td>Programmer</td> <td>BFSB, DIR, NINCDS</td> </tr> </table>			PI: Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS	Others: Sylvia Edelstein	Chief, Data Processing Sec.	BFSB, DIR, NINCDS	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS	Deborah G. Hirtz, M.D.	Pediatric Neurologist	DNB, DCDND, NINCDS	Dolores Jones	Computer Assistant	BFSB, DIR, NINCDS	Mary F. Livingston	Clinical Data Assistant	DNB, DCDND, NINCDS	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINCDS	Jack Panossian	Programmer	BFSB, DIR, NINCDS
PI: Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS																								
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Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS																								
Deborah G. Hirtz, M.D.	Pediatric Neurologist	DNB, DCDND, NINCDS																								
Dolores Jones	Computer Assistant	BFSB, DIR, NINCDS																								
Mary F. Livingston	Clinical Data Assistant	DNB, DCDND, NINCDS																								
Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINCDS																								
Jack Panossian	Programmer	BFSB, DIR, NINCDS																								
COOPERATING UNITS (if any) Cerebral Palsy and Other Motor Disorders Section, DNB, DCDND, NINCDS; University of Washington																										
LAB/BRANCH Biometry and Field Studies Branch																										
SECTION Mathematical Statistics Section																										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																										
TOTAL MAN-YEARS 3.0	PROFESSIONAL: 1.4	OTHER: 1.6																								
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Biometry and Field Studies Branch is the Statistical Coordinating Center for the ongoing clinical trial of the <u>behavioral and cognitive side effects of phenobarbital</u> used for <u>prevention</u> of <u>febrile seizure recurrence</u>. The accrual of patients has been completed, with 367 patients on study. Follow-up with psychometric testing will continue through FY '89. During this fiscal year, one interim analysis of the data was performed for evaluation by the Performance and Safety Monitoring Committee. In addition, extensive data editing is continuing, creation of structured files for analysis is completed, the distributions of the outcome variables have been examined and strategies are being developed for final analysis including the development of statistical methods for the analysis of language development data, play maturity data, activity level, and the recurrence rate of febrile seizures. *[This study supports the DNB/DCDND/NINCDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINCDS, and the contractor of the study is the University of Washington.]																										
14 - BFSB/DIR																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02593-05 BFSB															
PERIOD COVERED October 1, 1986 through September 30, 1987																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Stroke Data Bank																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 60%;">Mary A. Foulkes, Ph.D. Mathematical Statistician</td> <td style="width: 30%; text-align: right;">BFSB, DIR, NINCDS</td> </tr> <tr> <td style="vertical-align: top;">Others:</td> <td>Kathryn H. Chantry Statistician</td> <td style="text-align: right;">BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Gary Kamer Programmer</td> <td style="text-align: right;">BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Margaret Meadows Statistician Asst.</td> <td style="text-align: right;">BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Brenda Dyer Programmer</td> <td style="text-align: right;">BFSB, DIR, NINCDS</td> </tr> </table>			PI:	Mary A. Foulkes, Ph.D. Mathematical Statistician	BFSB, DIR, NINCDS	Others:	Kathryn H. Chantry Statistician	BFSB, DIR, NINCDS		Gary Kamer Programmer	BFSB, DIR, NINCDS		Margaret Meadows Statistician Asst.	BFSB, DIR, NINCDS		Brenda Dyer Programmer	BFSB, DIR, NINCDS
PI:	Mary A. Foulkes, Ph.D. Mathematical Statistician	BFSB, DIR, NINCDS															
Others:	Kathryn H. Chantry Statistician	BFSB, DIR, NINCDS															
	Gary Kamer Programmer	BFSB, DIR, NINCDS															
	Margaret Meadows Statistician Asst.	BFSB, DIR, NINCDS															
	Brenda Dyer Programmer	BFSB, DIR, NINCDS															
COOPERATING UNITS (if any) Departments of Neurology: Boston U. Medical Center, Michael Reese Hospital, Neurological Institute - Columbia University, and University of Maryland																	
LAB/BRANCH Biometry and Field Studies Branch																	
SECTION Computer Applications Section																	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.6	OTHER 0.4															
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																	
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The Stroke Data Bank is a prospective observational study collecting data on hospitalized newly diagnosed <u>stroke patients</u>, at four clinical centers. The four collaborating clinical centers are responsible for the collection of acute care and longitudinal follow-up information using common definitions and procedures, under contracts N01-NS-2-2302, 2398-9, N01-NS-5-2384. The general objective for the project is to provide a large and comprehensive body of data for clinical research on the factors influencing <u>survival, morbidity and quality of life</u> following <u>onset of a stroke</u>. The BFSB serves as the statistical coordinating center for the project, providing an on-site front-end data entry system with interactive feedback for data editing; the data base management system for transmission, storage and retrieval of data, for monitoring of data acquisition and its quality; and for statistical collaboration with the clinical investigators for the analysis of the primary research questions. The project has completed its fourth and final year of data collection and has entered 1805 patients as of September, 1986. The first major analyses began after accrual of patients was completed (September, 1986) and the acute care data was entered and edited. A mathematical model for identifying patients at greatest risk for progression of stroke has been developed based on the Pilot Stroke Data Bank, validated and revised based on the Main Phase SDB. The characteristics of the diagnostic subtypes of lacunar and infarcts of undetermined cause have been examined in detail. Mathematical models for both infarcts and intracerebral hemorrhages have been developed to identify patients unlikely to survive for one month following their stroke. Patient and lesion characteristics detected on CT scan have been identified that are associated with silent or unreported old strokes.																	
15 - BFSB/DIR																	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02516-06 BFSB
PERIOD COVERED <u>October 1, 1986 through September 30, 1987</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Traumatic Coma Data Bank</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mary A. Foulkes, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS	
Others:	Kathryn H. Chantry Statistician BFSB, DIR, NINCDS Gary Kamer Programmer BFSB, DIR, NINCDS Margaret Meadows Statistician Asst. BFSB, DIR, NINCDS Brenda Dyer Programmer BFSB, DIR, NINCDS	
COOPERATING UNITS (if any) Depts. Of Neurology: Medical College of Virginia, University of California - San Diego, University of Texas - Galveston, University of Virginia		
LAB/BRANCH <u>Biometry and Field Studies Branch</u>		
SECTION <u>Computer Applications Section</u>		
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The <u>Traumatic Coma Data Bank</u> is a prospective observational study collecting data on <u>severely head injured</u> patients at four clinical centers. The four collaborating centers are responsible for the collection of acute care and longitudinal follow-up information using common definitions and procedures, under contracts N01-NS-3-2339-42. The research objectives for the project were formulated by a steering committee composed of the principal investigators from the clinical centers, other outside experts, and BFSB staff, with the concurrence of the BFSB Advisory Committee. The research objectives were the basis for determining the specific data to be collected, the format of the data collection forms and the data collection procedures. The general objective for the project is to provide a large and comprehensive body of data for clinical research on the factors influencing <u>survival and quality of life</u> following a severe head injury. The BFSB is the statistical coordinating center for the project, providing an on-site front-end data entry system with interactive feedback for data editing; the data base management system for transmission, storage and retrieval of data, for monitoring of data acquisition and its quality; and for statistical collaboration with the clinical investigators and statistical analysis oversight for the primary research questions of the data banks. The project is in its third year of data collection and has entered over 900 patients as of June, 1987. The first major analyses will begin after accrual of patients has been completed in September, 1987 and the acute care data has been entered and edited. The focus of the Branch during the data collection period will be on the monitoring of the project with regard to completeness and quality of data collection.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02590-05 BFSB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Studies on the Stroke Data Bank

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Mary A. Foulkes, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS

Other: James M. Dambrosia, Ph.D. Chief, Mathematical Statistics Section BFSB, DIR, NINCDS

COOPERATING UNITS (if any)

Departments of Neurology: Boston University Medical Center, Michael Reese Hospital, Neurological Institute-Columbia University, and University of Maryland

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.35

PROFESSIONAL

0.30

OTHER

0.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project currently includes three studies each of which is a component of the Stroke Data Bank or its precursor, the Pilot Stroke Data Bank. The studies are: (1) Evolving Stroke. Using demographic, history, clinical and laboratory data, this study describes the temporal course of stroke-in-evolution and attempts to identify factors that cause or contribute to evolution. (2) Prognostic factors for 30-day mortality. Multiple logistic regression models, one for ischemic stroke and another for intracerebral hemorrhage were used to determine prognostic factors for 30-day mortality. Logistic models were derived using data from the pilot project: 620 ischemic strokes with 52 deaths and 94 intracerebral hemorrhages with 32 deaths. Potential risk factors (112 in all) were initially screened by univariate statistical methods and those screened positive were examined multivariately in the logistic model. These derived models will be cross-validated by examination of their predictive ability on data from the current Stroke Data Bank. (3) Discrimination between intracerebral hemorrhage and ischemic stroke. This study identifies factors, excluding CT information, available shortly after stroke onset that provide optimal classification into the two diagnostic categories.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02586-05 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) An Examination of Multiple Cause of Death Data for Stroke*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Herbert M. Baum, Ph.D. Demographer BFSB, DIR, NINCDS (No longer at NIH)		
COOPERATING UNITS (if any) Center for Population Studies, Duke University		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Surveys and Demographic Studies Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.0	PROFESSIONAL: 0.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use stenderd unreduced type. Do not exceed the space provided.) This project has three main goals. First to determine whether a change in the coding of a stroke on death certificates as underlying versus as an associated cause of death is partially responsible for the large decline in the <u>rates of stroke mortality</u> as calculated from the underlying cause of death. Next, to <u>construct life tables</u> and approximate the impact of eliminating stroke as a cause of death; and lastly, to examine the pattern of <u>multiple causes of death</u> which occur from stroke. Computer tapes, issued by the National Center for Health Statistics, containing all death certificates in the United States for the period 1968-1978 were used. All certificates where stroke (ICDA-8 Codes 430-438) was listed as either an underlying or associated cause of death were selected for study. The data were then tabulated by age, race, and sex. Life tables were constructed to estimate the change in <u>life expectancy</u> if stroke were eliminated as a cause of death. An examination of disease pairs (underlying and associated) was also undertaken. During FY '84 an article on "CVD Mortality, 1968-1978; Observations and Implications" was published. Another article investigating the diseases which appear in conjunction with stroke on the death certificate entitled "Cerebrovascular disease mortality: The relationship of underlying and associated causes of death," has been submitted for publication. *[This project has been subsumed under: Intramural Statistical Collaboration and Consultation (Z01-NS-02652-03)]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02637-04 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Stroke and Trauma Program Phase I-II Studies of Stroke Therapies*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BFSB, DIR, NINCDS
Others: Sylvia Edelstein	Chief, Data Processing Section	BFSB, DIR, NINCDS
Robert Richter	Computer Systems Analyst	BFSB, DIR, NINCDS
COOPERATING UNITS (if any) Division of Stroke and Trauma, NINCDS		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 0.8	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This project includes all aspects of data coordination and management, for studies of <u>interventional therapies</u> for stroke initiated by task orders issued under the aegis of the DST Master Agreement. Currently seven studies, each with two to three clinical centers, are in various stages of operation. A dose escalation study of <u>Maloxone</u> with acute cerebral ischemia has been completed and based on maximum tolerated dose, toxicity, and adverse effects were established. A Phase II study of <u>Maloxone</u> was also completed and analysis files have been constructed. Data collection for a pilot study of the benefits of <u>hypervolemic hemodilution</u> (DEXTRAN-40) for the treatment of <u>stroke-in-evolution</u> has also been completed. A dose-escalation Phase II study of <u>Nicardipine</u>, a calcium channel blocker, for the prevention of vasospasm following subarachnoid hemorrhage, and Phase II studies of <u>Heparinoid</u>, <u>Nicardipine</u> and <u>rt-PA</u> for the treatment of acute cerebral ischemia are ongoing. Patient accrual and data collection for these studies will be completed by FY '88. *[This project supports the Division of Stroke and Trauma contract entitled: Cerebrovascular Clinical Research Master Agreement. The Project Officer is Dr. John Marler.]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02483-07 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Predictive Value of the EEG in Febrile Seizures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Sherrie E. Emoto, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS Others: Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINCDS Martha Griswold Statistician BFSB, DIR, NINCDS Deborah G. Hirtz, M.D. Pediatric Neurologist DNB, DCDND, NINCDS Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINCDS		
COOPERATING UNITS (if any) Cerebral Palsy and Other Motor Disorders Section, DNB, DCDND, NINCDS; Pediatric Clinic, University of Skopje, Yugoslavia (Nikola Sofijanov)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.40	PROFESSIONAL: 0.20	OTHER: 0.20
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This population based study will evaluate the significance of the <u>EEG</u> as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcome with respect to <u>febrile seizure</u> recurrence and <u>afebrile seizure</u> occurrence will be reported. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study is being carried out in Skopje, Yugoslavia, at the Pediatric Clinic of the University of Skopje. The study began in FY '82 and will be completed in FY '88. Patient accrual was completed in December, 1984, by which time approximately 400 patients with a febrile seizure, no prior complex or multiple seizures and with a normal or nonspecific abnormal EEG, were registered into the study and began the study protocol and follow-up. An additional 300 patients with a specific abnormal EEG were entered for baseline information and follow-up. Data monitoring, editing and file creation are continuing. Statistical analysis of baseline EEG and its association with characteristics of the child and the clinical characteristics of the seizure began in FY '87. Follow-up should be completed on all patients by FY '88.		
20 - BFSB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02411-09 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Survey of Practice in the Management of Febrile Seizures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Young Jack Lee, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS Others: Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINCDS Deborah G. Hirtz, M.D. Pediatric Neurologist DNB, DCDND, NINCDS Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINCDS		
COOPERATING UNITS (if any) Cerebral Palsy and Other Motor Disorders Section, DNB, DCDND, NINCDS		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.00
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A survey of clinical practice in the management of febrile seizures was conducted using the AMA membership list. The survey questionnaire was sent to a probability sample of 10,000 physicians. The primary data analysis has been completed. From the analysis, it was determined how each medical discipline manages children with febrile seizures. Medical factors that are, in themselves, considered sufficient for chronic treatment or consultation were determined for each medical specialty. Goals of treatment, preferred medicines, whether and why to monitor blood drug levels, and whether and why to hospitalize were analyzed for each specialty. In order to carry out the appropriate analysis, methods for adjusting the effects of concomitant variables for the categorical data analysis were evaluated. The logistic regression was selected as a principal method for the adjustment. The results of the survey indicate that the management of a child with febrile seizures may differ depending on the specialty of the attending physician. A paper reporting the major findings has been published by the <u>American Journal of Diseases of Children</u>. This study has been completed.		
21 - BFSB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 NS 02594-05 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Factors Predictive of Reading and Writing Skills in the Congenitally Deaf*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Richard F. Raubertas, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS
Others: Christy Ludlow, Ph.D. Judith Cooper, Ph.D.	Speech Pathologist Speech Pathologist	DCD, DIR, NINCDS DCD, DIR, NINCDS
COOPERATING UNITS (if any) Central Institute for the Deaf, St. Louis, MO (Ann Geers); Gallaudet College, Washington, D.C. (Donald Moores)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.2	PROFESSIONAL 0.1	OTHER 0.1
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project consists of the statistical and data management aspects of a Communicative Disorders Program contract. Tasks include design of data collection and monitoring procedures, and statistical analysis of study data. The study will examine factors that may be associated with development of <u>reading and writing skills in the congenitally deaf</u>. Study subjects comprise three groups of deaf 16- to 17-year-olds, with 65 subjects in each group. Each group includes only subjects who received their preschool language training through one of three approaches: aural-oral, total communication, and American Sign Language. Data are collected on the audiologic, familial, and educational background of the subjects, and on their present language skills. These data will be examined for their association with present reading and writing skills of the subjects. A pilot study has been completed and the main data collection phase is now in progress. *[This project is the BFSB/NINCDS support of the DCD contract study NIH-NINCDS-84-19. The project officer is Dr. Christy Ludlow, DCD, NINCDS.]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02638-04 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Survey of Major Neurological Disorders in Copiah County, Mississippi		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Dallas W. Anderson, Ph.D.	Mathematical Statistician BFSB, DIR, NINCDS
Other:	Bruce S. Schoenberg, M.D., Ph.D. Chief	NEB, DIR, NINCDS
COOPERATING UNITS (if any) University of Mississippi Medical Center, Jackson, MS (Armin F. Haerer)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.50	0.40	0.10
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) To determine the prevalence of frequently occurring neurologic disorders in a biracial population, a survey of households and chronic care institutions was carried out for all residents of Copiah County, MS. Along with a complete census, interviews were held using extensive questions about diagnoses, signs, and symptoms of neurologic disorders. Over 97% of eligible households participated, comprising 23,842 persons (49% black, 50% white, 1% other). Persons with responses suggesting one or more of these disorders were examined by a neurologist who used defined diagnostic criteria. Age-adjusted prevalence ratios for cerebral palsy, epilepsy, stroke, and severe dementia were somewhat higher in blacks than in whites, while the age-adjusted prevalence ratio for essential tremor was slightly higher in whites. For Parkinson's disease, there was no difference in age-adjusted prevalence ratios between the races. With respect to particular disabilities in persons with the disorders mentioned above, it was found that proportionately more institutionalized men than institutionalized women were functionally disabled. Any other activities related to this project will be subsumed under the project: Intramural Statistical Collaboration and Consultation (Z01-NS-02652-03). This project is completed.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02505-07 BFSB												
PERIOD COVERED October 1, 1986 through September 30, 1987														
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Headache in Pregnant Women														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">Ta-Chuan Chen, Ph.D.</td> <td style="width: 30%;">Mathematical Statistician</td> <td style="width: 10%;">BFSB, DIR, NINCDS</td> </tr> <tr> <td>Others:</td> <td>Jonas H. Ellenberg, Ph.D.</td> <td>Chief</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>Sylvia Edelstein</td> <td>Chief, Data Processing Section</td> <td>BFSB, DIR, NINCDS</td> </tr> </table>			PI:	Ta-Chuan Chen, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS	Others:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS		Sylvia Edelstein	Chief, Data Processing Section	BFSB, DIR, NINCDS
PI:	Ta-Chuan Chen, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS											
Others:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS											
	Sylvia Edelstein	Chief, Data Processing Section	BFSB, DIR, NINCDS											
COOPERATING UNITS (if any) Boston Children's Hospital (Dr. Alan Leviton)														
LAB/BRANCH Biometry and Field Studies Branch														
SECTION Office of the Chief														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS 0.45	PROFESSIONAL: 0.40	OTHER: 0.05												
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project investigates the relationship between <u>migraine headache</u> and other diseases based on the data collected from the large group of gravidae in the Collaborative Perinatal Project. Subgroups of women characterized by the absence and presence of migraine and other recurrent headaches prior to or during pregnancy, were identified. Characteristics of these subgroups were examined for a variety of demographic, sociological, medical and obstetric factors, as well as the association of headache with other disorders. Results indicate that pregnant women with a migraine history had higher rates of smoking and of other symptoms and illnesses than women without a migraine history, and the presence of smoking history was associated with the prevalence of other symptoms and illnesses. Although there is no evidence that cigarette smoking might trigger or exacerbate migraine attacks in these women, smoking might have an additive effect on the association of migraine conditions with heart and thrombotic diseases, some respiratory and allergic diseases and peptic ulcer. A manuscript has been accepted for publication. Children of mothers with a history of migraine appear to have higher incidence of seizures and some infectious and allergic diseases than children born to mothers in the non-migraine group. More intensive statistical analyses are being carried out to examine the apparent associations.														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 NS 02312-11 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Maternal Infection Study*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS
Other: John L. Sever, M.D., Ph.D.	Chief	IDB, DIR, NINCDS
Martha Griswold	Statistician	BFSB, DIR, NINCDS
Anita Ley	Microbiologist	IDB, DIR, NINCDS
Dorothy O'Neill	Clinical Nurse	IDB, DIR, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.06	PROFESSIONAL: 0.05	OTHER: 0.01
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Analysis of the Collaborative Perinatal Project (CPP) data continues in the area of <u>maternal infection</u>. (The CPP is a prospective study of approximately 60,000 gravidae and the <u>follow-up</u> of their <u>children</u> through the seventh year of life.) Two primary methodologies have been used, a prospective and a case control approach. Since serological confirmation of all of the common infections occurring during pregnancy on all women in the project would not have been feasible, a case control design has been implemented assessing the titer of 11 antigens in women with abnormal children, in comparison with matched control women with normal children. This study did not show any clear association between infection during the second and third trimester of pregnancy and specific damage to the child. A prospective study of the association of childhood morbidity with maternal antibody level to toxoplasmosis and a descriptive study of the distribution of titers and frequency of seroconversions by race and age of gravidae for various antigens in a population of pregnant women, are in progress or have been completed. The prospective serological study of toxoplasmosis and its relationship with pregnancy outcome, based on the first 23,000 pregnancies in the study, has shown increases in the risk of deafness, microcephaly and low IQ among children born to women with high maternal antibody to toxoplasmosis. Manuscripts reporting the results of the toxoplasmosis study and the study of serologically confirmed infections have been submitted for publication. *[This study is the BFSB/NINCDS portion of a larger study entitled: Perinatal Infections Causing Damage to the Child - Collaborative Perinatal Project, 201 NS 00402-31 ID. The principal investigator on the overall study is Dr. John L. Sever, Chief, IDB, DIR, NINCDS.]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02497-07 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Indo-U.S. Study of Head Injury (Phase I)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINCDS		
COOPERATING UNITS (if any) University of VA Dept. of Neurosurgery, Charlottesville, VA All-India Institute of Medical Science, New Delhi, India		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.00	PROFESSIONAL: 0.00	OTHER: 0.00
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) For Phase I of this study, information on <u>head-injured persons</u> has been collected in independent research efforts in Charlottesville, Virginia, and in New Delhi, India. A preliminary review of these data collection efforts indicated significant overlap in the type of information collected. Final analysis has identified differences and similarities in the etiology, treatment and outcome of these head-injured populations, but has indicated that prospective observational studies or clinical trials on head injured patients is feasible. The report on Phase I of the study has been prepared, and will be submitted for publication.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02651-03 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Senile Dementia Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Frances M. Baker, M.D., M.P.H. Psychiatrist/ Epidemiologist BFSB, DIR, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.15	PROFESSIONAL: 0.15	OTHER: 0.00
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In conjunction with the Baltimore site of the Epidemiologic Catchment Area Survey of NIMH, NINCDS funded a detailed dementia workup on those persons in the elderly sample identified with dementing illness, by comprehensive psychiatric examination. The detailed dementia workup included a neurologic examination and comprehensive laboratory studies which included thyroid function tests, electrolytes, BUN and glucose, B12 and folate levels, calcium and phosphorous levels, syphilis serology, urinalysis, chest X-ray, EKG, EEG, and CT scan. The foci of this investigation were (1) to explore the limitations of the Mini-Mental-State-Examination (MMSE) as a screening instrument, (2) to explore which components of the comprehensive dementia workup provide the highest specificity when used as a dementia screening test, (3) to develop a screening model for dementia with a sensitivity and specificity improved beyond that of the MMSE, and (4) to examine the usefulness of this model in other elderly populations. In addition, for the confirmed cases of dementia, additional information was gathered to assess the social and economic impact upon the caregivers. A review of the data indicated that only 36 confirmed cases of dementia were identified and available for further analysis. The sample size was not adequate to address the four areas of interest and to develop a model for dementia screening beyond the MMSE. After review of the information on social and economic impact, it was determined that the study of the burden on caregivers was also not feasible. A report of the detailed dementia workup of the 36 cases is being prepared.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02494-07 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Prevalence of Multiple Sclerosis in Colorado		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Herbert M. Baum, Ph.D. Demographer Others: None BFSB, DIR, NINCDS (No longer at NIH)		
COOPERATING UNITS (if any) The Rocky Mountain Multiple Sclerosis Center, University of Colorado School of Medicine		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Surveys and Demographic Studies Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Rocky Mountain Multiple Sclerosis Center is one of a few centers devoted solely to the care of patients with multiple sclerosis, and is the only center of its type in the State of Colorado. Using records from the Center, the local chapter of the National Multiple Sclerosis Society, hospital records, and physician records we estimated the <u>prevalence of multiple sclerosis</u> for Weld and Larimer Counties, after accounting for duplicate cases. Crude point prevalence for the two-county region was 84 per 100,000. Methodological results revealed that the highest yield sources of cases were the MS service organizations and the neurology practice chart reviews. Prevalence surveys which neglect these sources may underestimate MS prevalence by as much as 20 to 40%. A manuscript "Higher than expected prevalence of multiple sclerosis in Northern Colorado: Dependence on methodologic issues," has been accepted for publication. No further work is planned by BFSB on this project and this project is considered completed.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02504-07 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Epidemiological Study of Pain*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Ta-Chuan Chen, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this project is to evaluate the overall and <u>age-specific incidence rates</u> of various <u>chronic pain syndromes</u> by developing a statistical technique to estimate incidence rates from age of onset data. The technique developed uses available estimates of age of onset data for headache and approximates incidence rates by superimposing the age of onset rates onto the age distribution of the given population. The incidence rates of disabling and/or severe headache were evaluated with data obtained from a Midwest non-clinical population survey. The validity of this procedure was evaluated by comparing the results with the incidence of disabling headache estimated from the British Second National Study of Morbidity Statistics. A report on this work has been prepared and will be submitted for publication. *[This project has been subsumed under: Intramural Statistical Collaboration and Consultation (Z01-NS-02652-03.)]		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02517-06 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Statistical Methodology for the Measurement of Pain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Ta-Chuan Chen, Ph.D. Mathematical Statistician BFSB, DIR, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has investigated the statistical problems involved in the measurement of experimental and clinical pain. (1) A study has been conducted to investigate the statistical technique used in deriving psychophysical measurements of pain. A report has been prepared for this work dealing with the interrelationship of sensory-decision-theory measures such as d' and Beta and nonparametric measurement indices, such as p(A), Hodo's percent bias and MacNicol's index of response bias, Beta. The investigation of this part of the work is completed. The report of this study was presented at the 4th World Congress on Pain in 1984. (2) A study of statistical quantification of the temporal characteristics of persistent, episodic pain such as migraine headache using a semi-Markov model has been investigated. An appropriate clinical data set on which to test this model is currently unavailable and no further work is planned in this area until such a data set is obtained. This study is completed.		
30 - BFSB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02654-02 BFSB
PERIOD COVERED March 15, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Incidence of Mental Retardation (MR) in Olmstead County		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Frances M. Baker, M.D., M.P.H.	Psychiatrist/ BFSB, DIR, NINCDS Epidemiologist
Other:	Bruce S. Schoenberg, M.D., Ph.D.	Chief NEB, DIR, NINCDS
COOPERATING UNITS (if any) Neuroepidemiology Branch, DIR, NINCDS; Mayo Clinic, Statistical Section (Leonard Kurland, M.D., Chief)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In conjunction with Dr. Leonard Kurland of the Mayo Clinic in Rochester, Minnesota, the birth records and school records of all Olmstead County residents born in 1960 and 1961 will be reviewed. All persons with mental retardation will be identified from these sources. <u>Mental retardation</u> (MR) is defined for the purpose of this study, as a fixed cognitive deficit which occurred before age 12 with some impairment of social adaptation. In addition to the specification of the incidence and identification of risk factors for MR in this birth cohort, <u>service utilization</u> by the MR cases will be assessed. Specific services which will be assessed will include medical (psychiatric, neurologic, gynecologic, and surgical), social (group homes, sheltered workshops, and rehabilitative programs), and educational (special program or projects and institutional placements). The long-term follow-up of the "9-3" mentally retarded child will be described and mechanism for prediction of functional level in adulthood will be investigated.		
31 - BFSB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02719-01 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Psychiatric Symptoms in Alzheimer's Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Frances M. Baker, M.D., M.P.H.	Psychiatrist/ Epidemiologist BFSB, DIR, NINCDS
Others:	Vijay Chandra, M.D., Ph.D. Bruce S. Schoenberg, M.D., Ph. D.	Medical Officer Chief NEB, DIR, NINCDS NEB, DIR, NINCDS
COOPERATING UNITS (if any) Neuroepidemiology Branch, DIR, NINCDS; Mayo Clinic, Department of Neurology and Statistical Section (Leonard T. Kurland, M.D., Chief; Emre Kokman, M.D., Neurologist)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) With the consent of Drs. Emre Kokman, Vijay Chandra, and Bruce Schoenberg (co-investigators), all incident cases of Alzheimer's Disease (AD) identified between 1965 and 1974 were made available to the Principal Investigator for three studies on this sample of 268 cases of clinically diagnosed AD. All medical records as well as state hospital records and nursing home records will be reviewed in order to identify psychiatric symptoms and psychiatric disorders which occurred in these patients throughout their lifespan. These symptoms and disorders will be divided into those occurring pre- and post- the onset of AD. A second study which will require review of all hospitalizations to identify all episodes of deliria (nocturnal disorientation) which occurred, will classify these episodes as pre- or post- the onset of AD. A third study will require the identification of all patients with clinically diagnosed AD and a major depressive episode (MDE). These will serve as the cases in a case-control study assessing MDE as a precursor or predisposing factor for AD. The two sets of controls will be identified from 1) age, sex, and race-matched controls with AD and <u>no</u> prior history of a MDE and 2) "normal controls" defined as matched for age, sex, and race, who do not have AD.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02506-07 BFSB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antibody Titers in Macacas on Cayo Santiago		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: James M. Dambrosia, Ph.D. Deputy Chief BFSB, DIR, NINCDS Other: William T. London, D.V.M. Acting Chief, Animal Health & Care Section DIR, NINCDS		
COOPERATING UNITS (if any) Animal Health and Care Section, DIR, NINCDS; Caribbean Primate Research Center, University of Puerto Rico (Matthew J. Kessler, Project Director)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20892		
TOTAL MAN-YEARS 0.05	PROFESSIONAL 0.05	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project will test for the presence of several viral antibodies in <u>adult and juvenile Macacas</u> on Cayo Santiago, Puerto Rico. This has been a closed colony of Rhesus monkeys since 1938. A serological screen carried out in the early 1950's indicated the presence of antibody to SV 40 (46%), <u>herpes B</u> (27%) and <u>measles virus</u> (80%) of the animals in the colony. The objective will be to determine after some 40 years as a closed colony, if herd immunity to the three previously studied antigens has been lost and would, therefore, provide an animal population useful for the testing of related strains of viruses. Three other antigens are also included in the screen. They are: rhesus CMV, simian retrovirus D (SRV-I) and simian T-lymphotropic virus type III (STLV-III). If this colony is shown to be serologically negative for these other three simian viruses, then animals from this colony could be used in the study of these simian diseases as models for related human diseases. With the tremendous interest and need for animal models of AIDS, we have added the following viral antigens to our testing protocol: human T-lymphotropic virus I (HTLV-I) and human immunodeficiency virus (HIV). In addition, we have broadened the scope of our testing to include animals in the NINCDS breeding colony in Puerto Rico. Animals shown to be serologically negative for antibodies to the added retroviral antigens are urgently needed for the Institute studies in AIDS.		
33 - BFSB/DIR		

ANNUAL REPORT

October 1, 1986 through September 30, 1987
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1986 through September 30, 1987
Developmental and Metabolic Neurology Branch, DIR
National Institute of Neurological and Communicative Disorders and Stroke

Roscoe O. Brady, M.D., Chief

Principal activities of the Branch concern the following areas of investigation: 1. Sphingolipid and mucopolysaccharide synthesis, catabolism, and elucidation of enzymatic abnormalities in human metabolic disorders. 2. Clinical studies of neurogenetic diseases. 3. Development of therapy for patients with heritable diseases. 4. Production of cellular and animal models of disorders of metabolism. 5. Elucidation of the molecular basis of lysosomal storage disorders. 6. Development of gene transfer vectors and exploration of gene replacement strategies. 7. Role of gangliosides as modulators of cell growth. 8. Regulation of hormone-responsive adenylate cyclase messenger systems.

I. HEREDITARY METABOLIC DISORDERS

A. Type C and Type D Niemann-Pick Disease.

We have applied the discovery of the metabolic defect in Type C Niemann-Pick disease, impaired esterification of exogenous cholesterol, to the first prenatal diagnosis of this disorder. We have made important advances in our knowledge regarding the regulation of intracellular cholesterol trafficking. Patients with Type D Niemann-Pick disease, a genetic isolate in Nova Scotia, have been shown to have a derangement of intracellular cholesterol disposition closely similar to that in Type C Niemann-Pick disease. However, there are subtle differences between these phenotypes in alterations of cholesterol regulatory processes such as the down-regulation of LDL receptors on the plasma cell membrane, the down-regulation of HMGCoA reductase, and the up-regulation of acyl-CoA acylcholesterol transferase in these Niemann-Pick variants.

II. CLINICAL INVESTIGATION OF NEUROGENETIC DISEASES

A. A number of patients with previously undiagnosed neurologic disorders were seen on the clinical service during the year and correct diagnoses were established. Novel presentations of previously identified heritable metabolic disorders were documented, and where appropriate, case descriptions were reported. In particular, a patient with cholesteryl ester storage disease and splenic infarction resembling Gaucher's disease was diagnosed and the unusual features exhibited by this patient have been described and submitted for publication.

B. Patients with diurnal dystonia have been admitted to the ward and

treated. Their remarkably beneficial responses to L-DOPA are being critically evaluated.

C. We have extended our monitoring the effectiveness of Tegretol for the painful acroparesthesias of Fabry's disease and have become increasingly convinced that this appears to be the treatment of choice for these debilitating manifestations of this disorder.

III. ENZYME REPLACEMENT THERAPY

We have obtained long-term hematologic and symptomatic improvement in a young patient with Gaucher's disease treated with placental glucocerebrosidase whose oligosaccharide residues terminate with the mannose to target the enzyme to cells of the monocyte/macrophage system. We have obtained a consistent reduction in liver and erythrocyte-associated glucocerebroside in patients who received the enzyme. The study will be expanded to determine clinical effects of enzyme replacement on Gaucher patients.

IV. MODELS OF HUMAN LYSOSOMAL STORAGE DISORDERS

A. Biochemical Models of Gaucher's disease.

1. A rodent model of Gaucher's disease has been produced using ^{14}C -L-glucosylceramide, a non-metabolizable analog of glucocerebroside. Studies with this material revealed that the labeled material was rapidly cleared from hepatocytes after intravenous administration to rodents. A significant portion of this material was excreted in the bile. The analog also appeared in Kupffer cells where it had an extraordinarily long residence. In keeping with this observation, no evidence was obtained for the intercellular transfer of ^{14}C -L-glucosylceramide from Kupffer cells to hepatocytes. This disposition closely resembles the exclusive storage of glucocerebroside in Kupffer cells in patients with Gaucher's disease. A particularly interesting aspect of these experiments was the demonstration that ^{14}C -L-glucosylceramide was eventually cleared from Kupffer cells by a novel currently unknown mechanism. It is conceivable that Kupffer cells that store the analog eventually are turned over *in situ*. This observation is consistent with the discovery of reduction of Gaucher cells in the bone marrow many months following bone marrow transplantation in a patient with Gaucher's disease. Elucidation of this mechanism has important implications for the enzyme replacement currently underway in the Branch and suggests that the glucocerebroside-storing cells in various organs may eventually be cleared with proper therapy.

2. We have continued to explore the use of conduritol B epoxide, a non-competitive inhibitor of glucocerebrosidase for the development of a rodent analog of Gaucher's disease. By varying the injection schedule of this compound, we have achieved the largest accumulation of glucocerebroside in an experimental animal so far reported. We shall examine additional measures using this inhibitor to produce a model of Gaucher's disease so that enzyme replacement may be expeditiously examined in a laboratory setting.

B. Pharmacological Model of Hurler's disease.

Administration of the antitrypanosomal agent Suramin has been shown to induce pathological and biochemical responses in laboratory animals that strongly resemble hereditary mucopolysaccharide storage disorders in humans. This model has been explored in depth with regard to the reversibility of the abnormal chemistry and pathology that attend the accumulation of glycosaminoglycans (GAGs) induced by Suramin. It has been found that GAGs were maximally elevated between 10 and 18 days following a single dose of Suramin. Thereafter, the quantity of the GAGs gradually decreased. They had returned to normal by six months in the liver, spleen and lung, but remained elevated in the kidney. Morphological evidence of recovery was also seen by six months except for pathological changes in the lung. These findings provide a reasonable timetable for anticipating clinical responses in mucopolysaccharidosis patients receiving various therapeutic modalities such as bone-marrow transplantation, enzyme, or gene replacement.

C. Bone Marrow Transplantation in Canine Hurler's Disease.

The Branch has continued a collaborative study with investigators at the University of Tennessee with a colony of spontaneously mutated Plott hounds that resemble mucopolysaccharidosis Type I (Hurler's syndrome) in humans. Bone-marrow transplantation has been performed on affected animals and the biochemical responses were monitored by DMNB. A major finding of this work was that there were major corrective changes in the concentration of mucopolysaccharides in the CNS of the transplanted animals. This finding has extraordinary important implications for the treatment of humans with Hurler's syndrome and other metabolic storage disorders by bone-marrow transplantation since most investigators did not believe that such an approach would lead to beneficial changes in the nervous system.

V. MOLECULAR GENETICS OF LYSOSOMAL STORAGE DISORDERS

Sphingomyelinase, the enzyme lacking in patients with Types A and B Niemann-Pick disease, has been purified to homogeneity and polyclonal antibodies have been raised against the pure protein. This is a significant accomplishment concerning Niemann-Pick disease since we are now in position to identify molecular changes that occur in sphingomyelinase in this disorder and to clone the gene for this enzyme. Accurate gene mapping and enzyme replacement may now be considered for this disorder.

VI. GENE TRANSFER TECHNOLOGY

Retroviral vector systems have been developed and the transfer of the human β -globin gene and its regulated expression have been demonstrated. The gene for glucocerebrosidase, the enzyme involved in Gaucher's disease, is being packaged in high-titer recombinant viruses and its expression in bone marrow stem cells will be investigated. These experiments should lead to the development of transgenic mice, a major step toward obtaining critical information leading to gene replacement in humans with sphingolipid storage disorders.

VII. ROLE OF GANGLIOSIDES IN THE REGULATION OF CELL GROWTH

Important progress has been made in assessing the role of gangliosides on the control of cellular proliferation. The B subunit of cholera toxin which binds specifically to ganglioside GM₁ on cell membranes was shown to stimulate mitogenesis in quiescent rat thymocytes. On the other hand, rapidly proliferating cells can be made to reduce their growth activity upon exposure to the B subunit of cholera toxin. This process does not appear to involve adenylate cyclase, changes in cAMP levels, inositol phosphate metabolism, alteration of intracellular pH, or activation of protein kinase C. The control appears to be closely linked to modification of intracellular Ca²⁺. Current work is directed towards elucidating the mechanism involved in the ganglioside-mediated alteration of intracellular Ca²⁺.

VIII. REGULATION OF HORMONE-RESPONSIVE ADENYLATE CYCLASE

A. In contrast with the apparent absence of involvement of adenylate cyclase in the ganglioside-mediated transmembrane signalling indicated in the previous section, there are a number of hormones known to exert their regulatory effects by altering intracellular cAMP. Using specific types of cells with muscarinic receptors, it has been shown that these receptors can utilize either the adenylate cyclase system or the stimulation of phosphoinositide breakdown mechanism for signal transduction.

B. Other studies have resulted in the solubilization of dopamine D-1 receptors from rat striatal membranes. These receptors were shown to be coupled to G proteins of the adenylate cyclase system. The receptors have been reconstituted in phospholipid vesicles where both high- and low-affinity binding can be manipulated.

C. An important investigation has been undertaken concerning gonadotropin receptors that are also coupled to adenylate cyclase. The receptor appears to be composed of two subunits one of which is presumed to be hormone-binding and the other non-hormone-binding. Exposure to the hormone causes desensitization and changes in the hydrodynamic properties of the receptor. These findings implicate an alteration in the physical state of the receptor that is linked to its uncoupling from the adenylate cyclase system.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Division of Intramural Research, NINCDS
October 1, 1986 through September 30, 1987

Contractor: WEIZMANN INSTITUTE OF SCIENCE (N01-NS-3-2349)

Title: Production of Radiolabeled Glycolipids and Other Sphingolipid Derivatives.

Contractor's Project Director: Ora Goldberg, Ph.D.

Current Annual Level of Support: \$76,133

Objectives: To prepare glucocerebroside, sphingomyelin, and ceramidetrihexoside labeled with ^{14}C in critical portions of the molecule for diagnostic tests for Gaucher's disease, Niemann-Pick disease, and Fabry's disease.

Major Findings: The Weizmann Institute of Science has extensive and recognized expertise in the chemical synthesis of sphingolipids. Procedures have been developed to incorporate radioactive carbon- ^{14} into specific portions of sphingolipid molecules. These compounds are used to diagnose patients with the sphingolipid storage disorders listed above, to identify heterozygous carriers of these conditions, to diagnose these disorders prenatally, and to monitor enzyme isolation procedures for glucocerebrosidase, sphingomyelinase, and ceramidetrihexosidase.

Significance to Biomedical Research and to the Program of the Institute: The ability to diagnose patients, identify heterozygotes, and monitor pregnancies at risk for sphingolipid storage disorders represents major contributions to the control of the incidence of these diseases. These procedures are in wide use at the present time.

Proposed Course of the Contract: The contractor will provide radioactive sphingolipids necessary for diagnostic tests and for enzyme purification procedures. Analogues of sphingolipids will be prepared for the development of animal models of the human disorders. Sphingolipid derivatives will be synthesized for use as ligands in affinity column chromatography to expedite and improve the isolation of sphingolipid hydrolases.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Division of Intramural Research, NINCDS
October 1, 1986 through September 30, 1987

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-3-2351)

Title: Preparation of Glucocerebrosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: \$409,094

Objectives: To isolate human placental glucocerebrosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Gaucher's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental glucocerebrosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Gaucher's disease. The intravenous infusion of this enzyme causes a decrease in the quantity of glucocerebroside stored in the liver and in association with erythrocytes in the blood. Long-term administration of the enzyme has caused significant hematologic improvement in a patient with Gaucher's disease.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. The results indicated in the preceding paragraph will be extended leading to an unprecedented accomplishment regarding the treatment of human genetic diseases.

Proposed Course of the Contract: We are investigating procedures to stabilize and target the enzyme to the specific cells in which toxic quantities of lipid accumulate. We shall also continue to attempt to develop methods to deliver the enzyme to the central nervous system for the treatment of patients with the neuropathic forms of the disorder.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Division of Intramural Research, NINCDS
October 1, 1986 through September 30, 1987

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-3-2346)

Title: Preparation of Ceramidetrihexosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: \$104,005

Objectives: To isolate human placental ceramidetrihexosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Fabry's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental ceramidetrihexosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Fabry's disease. We are developing methods to target the enzyme to cells and tissues where ceramidetrihexoside is stored.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy for human diseases. If salutary clinical results can be obtained, an extraordinary milestone will have been accomplished regarding this type of a human genetic disease.

Proposed Course of the Contract: We have begun experiments to increase the delivery of this enzyme to specific cells where ceramidetrihexoside accumulates. We are reinitiating enzyme replacement trials in patients with Fabry's disease. We shall examine the effectiveness of the enzyme with regard to clearance of accumulated ceramidetrihexoside in the liver, kidney and in the blood, and monitor clinical responses to this form of therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 00815-27 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Metabolism of Complex Lipids of Nervous Tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R. O. Brady, M.D., Chief,	DMN NINCDS
OTHERS:	P. G. Pentchev, Ph.D., Biochem.	DMN NINCDS
	A. E. Gal, Ph.D., Organic Chemist	DMN NINCDS
	R. R. O'Neill, Ph.D., Staff Fellow	DMN NINCDS
	T. Tokoro, M.D., Vis. Fellow	DMN NINCDS
	J. M. Quirk, Biochemist	DMN NINCDS
	M. Comly, Biologist	DMN NINCDS
	H. S. Kruth, Ph.D., Sen. Invest.	EA NHLBI
COOPERATING UNITS (if any) Laboratory of Experimental Atherosclerosis, NHLBI		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 1. The metabolic defect in patients with <u>Type D Niemann-Pick disease (Nova Scotia variant)</u> has been shown to be due to <u>abnormal intracellular cholesterol homeostasis</u> that resembles, but differs in some respects, from the abnormality in <u>Type C Niemann-Pick disease</u> previously discovered by this Branch. The molecular lesion in these disorders results in: (1) failure to down-regulate LDL receptors on cell membranes; (2) lack of down-regulation of HMGCoA reductase, a key enzyme in cholesterol biosynthesis; and (3) inability to up-regulate acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the esterification of intracellular cholesterol. Based on this information, we have developed tests for the <u>diagnosis of Types C and D Niemann-Pick disease</u> and the <u>identification of heterozygotes</u>. In FY 87 these procedures were shown to be reliable for the <u>prenatal diagnosis</u> of these conditions. 2. Other work has centered on the synthesis and use of non-metabolizable analogs of glucocerebroside and galactocerebroside to examine the pathogenesis of <u>Gaucher's disease</u> and <u>Krabbe's disease</u> by developing rodent models of the respective human disorders. A novel analog of glucocerebroside has been synthesized and appears to be useful for the selection of mutagenized cells that lack glucocerebrosidase, the enzyme lacking in Gaucher's disease. These cells will be used to construct a <u>transgenic murine analog</u> of Gaucher's disease.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01309-22 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: P. H. Fishman, Ph.D. OTHERS: S. Spiegel, Ph.D. G. Matyas, Ph.D. R. O. Brady, M.D. C. Panagiotopoulos	Chief, Membrane Biochemistry Section Visiting Fellow Staff Fellow Branch Chief Guest Researcher	DMN, NINCDS DMN, NINCDS DMN, NINCDS DMN, NINCDS DMN, NINCDS
COOPERATING UNITS (if any) Laboratory of Cellular Metabolism, NHLBI (J. Moss; B. Hom); Laboratory of Kidney and Electrolyte Metabolism, NHLBI (J. S. Handler); Laboratory of Molecular Biology, NCI (S. A. Aaronson); FIDIA Research Laboratories, Italy		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Membrane Biochemistry Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.9	PROFESSIONAL: 2.4	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Gangliosides</u> appear to be important recognition molecules on the <u>cell surface</u> and have been implicated as <u>receptors</u> for certain bacterial toxins and <u>viruses</u>. Little is known, however, about the normal physiological role(s) of these <u>plasma membrane</u> components. To explore the role of surface gangliosides in cell growth, we used the B or binding subunit of <u>cholera toxin</u> as a specific probe for surface GM1. The only known function of the B subunit, which is multivalent, is to bind to the oligosaccharide chain of GM1. Exposure of quiescent rat thymocytes and mouse 3T3 cells to the B subunit resulted in a proliferative response as measured by increased DNA synthesis and cell numbers. The B subunit potentiated the effects of other mitogens such as epidermal growth factor, insulin and serum. As mitogens mediate their effects through several transmembrane signalling systems and induce the early expression of the proto-oncogenes <i>c-myc</i> and <i>c-fos</i>, the ability of the B subunit to initiate these early mitogenic responses was explored. The only acute response observed in both thymocytes and 3T3 cells exposed to the B subunit, was a rapid rise in intracellular free Ca^{2+} which depended on the presence of extracellular Ca^{2+}. There was no elevation of cAMP, inositol phosphates, or intracellular pH, or activation of adenylate cyclase or protein kinase C. The B subunit also induced expression of <i>c-myc</i> and <i>c-fos</i>. Although the mechanism by which the B subunit triggers a rapid increase in intracellular free Ca^{2+} and induction of proto-oncogene expression is not yet known, it must be through its specific binding to GM1 on the cell surface. Thus, gangliosides may play a role as membrane <u>transducers</u> of signals that regulate <u>cell growth</u>.		

9 DMN/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01457-21 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Chemical Synthesis of Radioactive Sphingolipids		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Andrew E. Gal, Ph.D., Chief, Neurochemical Meth. DMN NINCDS OTHER: Stephen P. Miller, Ph.D., Senior Staff Fellow DMN NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Sphingolipids containing radioactive isotopes were synthesized and used for metabolic studies and as diagnostic tools in sphingolipidoses. ¹⁴ C and ³ H labels were introduced by synthetic and semi-synthetic techniques, gas exposure and a new approach: functional group exchange. These techniques were used for the syntheses of radioactive enantiomorph derivatives of sphingolipids. These products are not metabolizable. Experimentation with these in animals creates "animal models" for metabolic diseases and opens new areas for biomedical studies.		

10 DMN/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02162-13 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synthesis of Compounds Analogous to Glycolipids		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Andrew E. Gal, Ph.D., Chief, Neurochemical Methodology Section OTHER: Stephen P. Miller, Ph.D., Senior Staff Fellow, Neuro-Chemical Methodology Section DMN, NINCDS DMN, NINCDS		
COOPERATING UNITS (if any) None.		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.9	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Work was continued on the syntheses of glycolipid analogues of sphingolipids that yield a chromogenic moiety on enzymatic hydrolysis. These compounds are used for the diagnosis and studies of <u>Niemann-Pick</u>, <u>Gaucher's</u> and <u>Krabbe's</u> disease. Conduritol B epoxide, a saccharide that strongly inhibits β-glucosidases, was synthesized by a method developed by this section that provides the product in greater yield than previously available and permits the preparation of this compound containing a tracer with extraordinarily high specific radioactivity. Administration of conduritol B-epoxide to animals produces a syndrome that resembles <u>Gaucher's disease</u> in humans by inhibiting the enzyme glucocerebrosidase. Radioactive conduritol B-epoxide was also synthesized by a novel method which allows the production of large quantities in highly active form of this product. It reacts with the active site of glucocerebrosidase isolated from normal human tissues and patients with <u>Gaucher's disease</u>. This use of the radioactive conduritol β-epoxide will materially accelerate the identification of the <u>amino acid substitutions (or deletions)</u> that occur in the glucocerebrosidase molecule in patients with <u>Gaucher's disease</u>. Also fluorescent and cytotoxic substrates were prepared which can be used for the separation of affected and normal cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02163-13 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Analytical Methods for the Use of Research of Sphingolipidoses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Andrew E. Gal, Chief, Neurochemical Methodology Section, DMN, NINCDS OTHER: Stephen P. Miller, Ph.D., Senior Staff Fellow, Neurochemical Methodology Section, DMN, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Neurochemical Methodology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>New analytical techniques</u> were developed and used in enzymatic research and in <u>clinical investigations of lipidoses</u>. The lipid content in human tissues, the diagnosis of lipid storage diseases by <u>gas, thin-layer chromatography</u> and other techniques were studied at the microgram level. The techniques we developed previously were improved, modified and used in connection with ongoing projects related to lipidoses in our laboratories and also as joint projects with outside groups. Numerous analytical studies were undertaken by using these techniques.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02366-09 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Hormone-Responsive Adenylate Cyclase		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: P. H. Fishman, Ph.D. OTHERS: R. V. Rebois, Ph.D. J. Baumgold, Ph.D. A. Sidhu, Ph.D. R. M. Bradley, B.S. M. A. Sullivan, B.S.	Chief, Membrane Biochemistry Section Senior Staff Fellow Special Expert Visiting Fellow Chemist Biologist	DMN, NINCDS DMN, NINCDS DMN, NINCDS DMN, NINCDS DMN, NINCDS DMN, NINCDS
COOPERATING UNITS (If any) Laboratory of Clinical Science, NIMH (J. Patel); Department of Biochemistry, University of Wyoming (T. H. Ji); Department of Pharmacology, Uppsala University, Sweden (L. Terenius, M. Olasmaa)		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Membrane Biochemistry Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 7.2	PROFESSIONAL: 3.7	OTHER: 3.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 1. Muscarinic receptors have been classified into M1- and M2-subtypes based on their affinity for the antagonist pirenzepine. We now show that both NG108-15 and SK-N-SH cells have M2 receptors. Yet, in the former cells, the receptors are coupled only to inhibition of adenylate cyclase; and in the latter cells, to stimulation of phosphoinositide breakdown. Thus, the same muscarinic receptor subtype can couple to two different signal transduction systems. 2. We have succeeded in solubilizing the D-1 dopamine receptor from rat striatal membranes and reconstituting it into phospholipid vesicles. The receptor remains functional and couples to G proteins as measured by agonist high affinity binding. The proportion of receptors in the agonist high affinity state is increased over 6-fold from that in the original membranes. This may have important implications for the physiological state of the D-1 receptor in striatal membranes and its ability to mediate dopaminergic responses. 3. The hydrodynamic properties of the gonadotropin receptor from MLTC-1 cells has been determined. These cells contain gonadotropin receptors coupled to adenylate cyclase. Under nondenaturing conditions, the Mr of the receptor is 130 kDa compared to 80 kDa under denaturing conditions. Thus, the receptor may be composed of hormone-binding and non-binding subunits. Following desensitization of the cells by choriogonadotropin, the hydrodynamic properties of the receptor changes; the receptor binds less detergent and exhibits a more elongated frictional ratio but retains the same Mr. Thus, desensitization appears to involve a physical change in the gonadotropin receptor which may promote its uncoupling from adenylate cyclase.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02435-08 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Mucopolysaccharidoses*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: George Constantopoulos, Ph.D., Research Biochemist, DMN NINCDS		
COOPERATING UNITS (if any) Robert M. Shull, D.V.M., Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN.		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The mucopolysaccharidoses (MPS) are a group of hereditary diseases characterized by defective metabolism of <u>glycosaminoglycans (GAGs)</u>. The disorders are usually associated with severe dysfunction of the <u>nervous system</u> as well as of <u>liver, spleen, heart, bone</u>, and other tissues. Objective of this project is the study of <u>mechanism of pathogenesis</u> of these diseases with emphasis on <u>brain involvement</u> and <u>mental retardation</u>. We are using a comparative approach. For this purpose we study the changes, in <u>GAGs, sphingolipids</u>, and pertinent <u>lysosomal enzymes</u> in tissues of patients with various types of <u>MPS</u> and we make <u>correlation</u> in terms of <u>clinical</u> and <u>ultrastructural</u> findings. Our laboratory contributed significantly in understanding the chemical pathology and in particular the neurochemistry of <u>MPS IH, MPS IS, MPS II, MPS III A and MPS III B</u>. To complement the studies with human subjects, a drug (suramin) induced <u>animal model of MPS</u> has been developed and a <u>canine model, (natural)</u>, of <u>MPS I (α-L-iduronidase deficiency)</u>, has been fully characterized. In an attempt to reverse the progressive deterioration caused by this incurable metabolic disorder, five dogs with <u>MPS I</u> were <u>transplanted with marrow</u> from normal or heterozygous littermates. <u>Transplanted bone marrow</u> provides the affected dogs with self-renewing source of cells that produce the enzyme needed to complete the metabolic process. One year after <u>transplantation</u> the results are very encouraging for this <u>neurodegenerative metabolic disorder</u> because we found marked <u>corrective changes</u> within the <u>central nervous system</u> of the dogs. *(Formerly: "Studies on the Mechanism of Pathogenesis of the Mucopolysaccharidoses")		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02453-07 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gaucher's Disease: Biochemical and Clinical Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Roscoe O. Brady, M.D., Chief, DMNB, DIR, NINCDS OTHERS: Norman Barton, M.D., Ph.D., Michele Filling-Katz, M.D., John Fink, M.D., Gregory Zirzow, B.S., Mark Garfield, M.S., H. Mankin, M.D., D. Rosenthal, M.D., and S. Doppelt, M. D., MGH, Boston, MA.; Philip Guzetta, M.D., CH, Washington, D. C.		
COOPERATING UNITS (if any) Dept. of Orthopaedic Surgery, Massachusetts General Hospital, Boston, MA; Children's Hospital, Washington, D. C.		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations & Therapeutics Section, Enzymology & Genetics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 5.5	PROFESSIONAL: 3.5	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Therapy for Gaucher's disease depends upon broad clinical and basic scientific knowledge of the disorder. Patients have been extensively studied and complications identified. Research on <u>glucocerebrosidase</u> addresses the <u>biochemistry</u>, <u>cell biology</u>, and <u>molecular genetics</u> of the enzyme. A <u>clinical trial</u> directed along the lines of pharmacodynamic assessment of mannose-terminated human placental glucocerebrosidase is underway. Results obtained in this study will form the basis of an efficacy investigation in Gaucher patients with this enzyme that is targeted to lipid storing cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS-02619-04 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Oxidative Metabolism in Patients with Inherited Neurological Diseases and in Mollicutes*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: George Constantopoulos, Ph.D., Research Biochemist, DMN, NINCDS		
COOPERATING UNITS (if any) Coriell Institute for Medical Research, Camden, New Jersey, and Surgical Neurology Branch, NINCDS		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An increasing amount of evidence points to a possible defect in <u>oxidative metabolism</u> in patients with certain inherited neurological disorders. Thus, a defect in the <u>pyruvate oxidation system</u> has been shown in some patients with lactic acidemia and diffuse neurologic disease, of the mitochondrial <u>malic enzyme</u> in patients with <u>Friedreich's ataxia</u>, and a partial deficiency of <u>glutamate dehydrogenase</u> in some patients with <u>olivopontocerebellar degeneration</u>. However, there is much controversy about the exact enzymatic defect(s). The objective of this project is the elucidation of the defect in some of these patients or in skin <u>fibroblasts</u> derived from such patients. For this purpose we are assaying a number of mitochondrial and non-mitochondrial enzymes in fibroblasts or leukocytes and we have initiated <u>electron microscopic studies</u> of the mitochondria. We became interested in the <u>mollicutes</u> because contamination of fibroblast cultures with mollicutes interfered with the assay of <u>pyruvate dehydrogenase complex</u> in these cells. The oxidative metabolism of mollicutes is poorly understood. Hopefully, the elucidation of the defect in these diseases will help in the diagnosis and therapeutic intervention in the patients. Knowledge of the physiology of mollicutes may help in understanding the pathogenicity of these organisms.		
*(Formerly: "Oxidative Metabolism in Patients with Inherited Neurological Diseases and In Mycoplasmas")		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02648-03 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological Modification of Human Glioma Cells In Vitro		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: George Constantopoulos, Ph.D. OTHERS: Roscoe O. Brady, M.D. Paul L. Kornblith, MMC, Dept. of Neurosurgery, Bronx, New York Research Chemist Chief DMN, NINCDS DMN, NINCDS		
COOPERATING UNITS (if any) Montefiore Medical Center, Bronx, New York 10467		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Shedding of various products from the cell surface of cancer cells</u> may have an important role in <u>loss of adhesion, metastasis, generation of immune-blocking factors</u> and other <u>pathophysiologic aspects of cancer</u> (Black PH, <u>NEJM</u>, 303:1315, 1980). <u>Human glioma cells</u> in tissue culture produce and shed in the media much greater amounts of <u>glycosaminoglycans (GAGs)</u> than normal <u>glial cells</u> (Glimelius et al <u>Biochem J</u>, 172: 443, 1978). <u>Glycosaminoglycans</u> are polyanionic compounds, usually bound covalently to a protein core. They are a prominent component of the cell surface and are implicated in cell-cell interaction. There is also evidence for existence of <u>hyaluronidase-sensitive protective coats</u> on some neoplastic cell lines including gliomas, and it has been suggested that the protective GAG coat may impede the <u>immune response</u> and the efficacy of <u>chemotherapy</u>. Our objective was to modify or prevent the synthesis and shedding of GAGs in human glioma cells in culture, and possibly to render them more susceptible to chemotherapy and more immunologically responsive. For this purpose we use, singly or in combination, the <u>differentiating agents dimethyl sulfoxide (DMSO), sodium butyrate, retinoic acid, and dexamethasone</u>. These agents are known to affect the synthesis of GAGs in other systems.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02657-03 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular and Genetic Studies of Niemann-Pick Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Norman Barton, M.D., Ph.D. OTHER: Katherine Oliver, Biologist CITS, DMN, CITS, DMN, NINCDS NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations and Therapeutics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: .9	PROFESSIONAL: 0.3	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Niemann-Pick disease is a progressively debilitating, neurogenetic disorder which is characterized biochemically by the accumulation of sphingomyelin in several tissues and organs in conjunction with deficiency of the lysosomal hydrolase, sphingomyelinase. Detailed description of various phenotypes in terms of cellular pathochemistry and molecular genetics has not been accomplished to date. A major obstacle in this area has been the absence of reproducible techniques for the isolation of homogeneous preparations of sphingomyelinase. Employing novel detergent and chromatography systems, we have purified sphingomyelinase to homogeneity. The purified enzyme migrates with an apparent molecular weight of 67,000 daltons in SDS-polyacrylamide gels under both reducing and nonreducing conditions. Kinetic analyses and determinations of the primary protein structure and carbohydrate composition are in progress as are efforts to develop and characterize monoclonal and polyclonal antibodies to the purified enzyme. Availability of well characterized antibodies will allow us to proceed to cloning of the gene for sphingomyelinase. Characterization of the phenotypes of Niemann-Pick disease in terms of protein polymorphisms and specific mutations at the DNA level will be undertaken.</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02664-03 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical Studies of Neurogenetic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Norman Barton, M.D., Ph.D. OTHERS: Roscoe O. Brady, M.D., John Fink, M.D., Mark Hallett, M.D., Jacob Sokol, M.D., Franki Merrick, M.D., Kamal Ishak, M.D., Ph.D., AFIP; Henry Mankin, M.D., and Samuel Doppelt, M.D., MGH, Boston, MA.		
COOPERATING UNITS (if any) Interinstitute Medical Genetics Program, C.C., NIH, Massachusetts General Hospital; Armed Forces Institute of Pathology		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations & Therapeutics Section/Enzymology and Genetics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
7.0	6.5	0.5
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The <u>clinical study of neurogenetic diseases</u> provides the context in which the goals of <u>improved diagnosis</u> and potential <u>treatment</u> modalities are identified. Several new <u>clinical phenotypes</u> have been recognized including a number of cases of hexosaminidase, a deficiency presenting as motor neuron disease, glycerol kinase deficiency presenting as acidemia and stupor without mental retardation, bipterin deficiency presenting as familial dystonia and a severe variant of cholesterol ester storage disease presenting with advanced liver disease. A number of <u>rare phenotypes</u> have also been identified including Tay-Sachs Disease in a young non-Jewish child, two cases of Juvenile Krabbe's Disease, an unusual presentation of multiple sulfatase deficiency, an unusual case of San Filippo-A Disease, a mild variant of Monquio's Syndrome, a case of arylsulfatase activation deficiency, a case of Menke's Disease in a female infant and an unusual variant of mitochondrial encephalomyelopathy. A large number of <u>typical neurogenetic diseases</u> have been confirmed by studies performed in this protocol. Studies of <u>chorionic villus</u> samples have allowed early prenatal diagnosis of certain lysosomal storage disorders. Clinical trials of enzyme replacement in Gaucher's disease, bipterin supplementation in diurnally variant dystonia and cholesterol restriction therapy in C and D <u>variants of Niemann-Pick Disease</u> are being conducted. The application of <u>gene transfer</u> to human disease is under consideration.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02730-01 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Retroviral Mediated Transfer of Human Globin Genes into Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: S. Karlsson, M.D., Ph.D., Visiting Associate, Molecular and Medical Genetics Section D. Bodine, Ph.D., Staff Fellow L. Perry, B. S., Biologist A. W. Nienhuis, M.D., Chief Th. Papayannopoulou, M.D., Dept. of Hematology University of Washington James Leonardo, Ph.D. DMN NINCDS CHB NHLBI DMN NINCDS CHB NHLBI DMN NINCDS		
COOPERATING UNITS (if any) Clinical Hematology Branch, NHLBI Dept. of Hematology, Univ. of Washington		
LAB/BRANCH Developmental & Metabolic Neurology Branch, NINCDS		
SECTION Molecular & Medical Genetics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Retroviral vectors</u> can be used to transfer genes effectively into cell lines and primary cells. Less is known, however, whether these vectors can be used to transfer genomic genes into tissue culture cells and animals to yield tissue specific expression of the <u>transferred gene</u>. We have now shown, that retroviral vectors carrying the human β-globin gene can lead to <u>regulated expression of human globin RNA and protein</u> in murine <u>erythroleukemia cells</u>. In addition, we have recently designed a new high titer ecotropic retroviral vector that has been used to transfer the human beta globin gene into CFU-S murine spleen colonies and long-term reconstituted mice. Expression of the human β-globin chain has been in the erythrocytes four weeks after injection and bone marrow transplantation.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02731-01 DMN
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Therapy of Inherited Enzyme Deficiencies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: OTHERS: S. Karlsson, M.D., Ph.D., Visiting Associate John Fink, M.D., Clin. Investigations & Therap. Section Leland Perry, Biologist, Molecular and Med. Gen. Sect. Roscoe O. Brady, M.D., Chief Mia Horowitz, Sen. Scientist, Weizmann Inst. of Science Israel DMN NINCDS DMN NINCDS DMN NINCDS DMN NINCDS		
COOPERATING UNITS (if any) Weizmann Institute of Science, Rehovut, Israel		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Molecular and Medical Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Gaucher's disease</u> is an inherited disorder caused by a mutation of the gene for the enzyme glucocerebrosidase. The normal gene has now been cloned by several laboratories and at least two have inserted the cDNA into <u>retroviral vectors</u> and obtained expression of the enzyme in cell lines from patients following gene transfer. However, retroviral vectors produced to a high titer and stability containing this gene have not been made yet. It is the aim of this project to design vectors that can generate <u>high titer helper-free recombinant viruses</u> that contain appropriate promoters and/or enhancers leading to expression of the glucocerebrosidase gene following <u>gene transfer</u> into bone marrow stem cells and embryonic stem cells. "Gene therapy" of <u>mice with the human glucocerebrosidase gene</u> and creation of <u>transgenic mice</u> using these vectors will help evaluate the feasibility and efficiency of retroviral gene therapy for Gaucher disease.		
21 DMN/DIR		

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Experimental Therapeutics Branch

National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1986 through September 30, 1987

Experimental Therapeutics Branch

National Institute of Neurological and Communicative Disorders and Stroke

Thomas N. Chase, M.D., Chief

The goal of the Experimental Therapeutics Branch is to develop improved pharmacotherapies for selected disorders of the central nervous system. An integrated program of fundamental and applied research seeks to define the relation between clinical signs of brain dysfunction and specific alterations in neuronal transmission; based on a detailed understanding of synaptic mechanisms and of potential sites for pharmacologic intervention, novel therapeutic approaches are developed to modify the affected systems and thus improve clinical function. In addition, molecular biologic approaches are being applied to the search for potential sites of pharmaceutical intervention in the regulation of genetic expression. In these efforts, Branch research at both clinical and preclinical levels remains focused on the dopamine system and selected peptidergic pathways in relation to dementing and extrapyramidal disorders.

The Experimental Therapeutics Branch is currently organized into four closely integrated components: Dr. David Sibley's recently initiated Molecular Pharmacology Unit has begun to carry out basic molecular biologic and pharmacologic studies of receptor mechanisms, especially involving the dopamine system; Dr. Judith Walters' Physiologic Neuropharmacology Section evaluates interactions between the dopamine system and other transmitter pathways within the basal ganglia; the Neuroendocrinology Unit, currently headed by Dr. Bibie Chronwall is studying molecular mechanisms which regulate neuropeptide synthesis; and Dr. Thomas Chase's Clinical Pharmacology Section explores pathogenetic mechanisms and pharmacologic interventions in Alzheimer's and Parkinson's disease and in closely related neurodegenerative disorders.

Biochemical Neuropharmacology Section/Molecular Pharmacology Unit

FY 87 represented a period of transition for the Biochemical Neuropharmacology Section which has now been converted to the Molecular Pharmacology Unit under the direction of Dr. David R. Sibley. Previously, under the guidance of Dr. John Keibabian in FY 86, the Biochemical Neuropharmacology Section focused its research on dopamine receptor pharmacology and pituitary cell biology. Various projects in FY 86 included the development of the D-1 selective dopamine receptor ligand [¹²⁵I] SCH 23982 and the development of techniques to permeabilize pituitary cells to examine mechanisms of hormone release. These projects have been continued into FY 87. [¹²⁵I] SCH 23982 has been used to characterize D-1 dopamine receptors via ligand binding and autoradiographic techniques in both the substantia nigra and retina of various species. Permeabilized pituitary cells have also been used to examine the role

of cAMP, ATP, and Ca^{2+} in regulating the release of α -MSH from the intermediate lobe of the pituitary gland.

Additional projects in FY 87 related to the Biochemical Neuropharmacology Section included a thermodynamic analysis of ligand interactions with D-2 dopamine receptors, an investigation of D-2 dopamine receptor desensitization, and a characterization of the effects of estrogen on pituitary dopamine receptor function.

In FY 87 and beyond, the Molecular Pharmacology Unit will continue investigating the biochemical and pharmacological properties of dopamine receptors but using more molecular approaches. A major thrust of the Unit's efforts will be to develop large-scale purification procedures for both D-1 and D-2 dopamine receptors. Once sufficient quantities of receptor protein are obtained, a variety of projects including the development of receptor antibodies, receptor phosphorylation and reconstitution, and molecular cloning of the receptor genes will be undertaken.

Neuroendocrinology Unit

Pharmacology and Cellular Biology of Peptidergic Neurons

The most recently identified and the major known class of neurotransmitters and hormones is comprised of peptides. The goal of the Unit has been to develop an understanding of the basic regulatory mechanisms in cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic systems. Two studies are ongoing. The first examines the regulation of biosynthesis pro-opiomelanocortin (POMC). The primary model under investigation is the POMC containing neuronal and endocrine system which secretes ACTH, α -MSH and β -endorphin. These peptides influence arousal and cognitive processes through interactions with central MSH receptors and analgesia through interactions with μ and Δ opioid receptors. The second investigation concerns the transcriptional regulation of neuropeptide Y in brain and the pheochromocytoma tumor cell line (PC-12).

1. Regulation of biosynthesis of POMC derived peptides.

Biosynthesis of peptides occurs in three steps. First, the process is initiated by signal transduction between cell surface receptors and the biosynthetic mechanisms. Second, peptide prohormone and processing enzymes are synthesized. Third, the final secretory form of the peptide is generated by cleavage and modification of the prohormone by post-translational processing enzymes.

In the last five years, the Unit investigated the third biosynthetic step -- the roles and regulation of post-translational processing of POMC. It was found that post-translational processing of the POMC-derived peptides dramatically changes their biological and behavioral activity. The POMC derived peptides, α -MSH and β -endorphin, bind to different postsynaptic receptors but have extensive interactions. In FY 84, it was found that the extent of post-translational processing of POMC was not only tissue specific but also varied in

different physiological situations. For example, it was observed that there are different ratios of POMC derived peptides in different tissues and this ratio changes in physiological situations. In FY 85 and 86, the Unit continued investigations of regulation of post-translational processing of POMC and post-translational processing enzymes. It was found that the biosynthesis of POMC and certain POMC post-translational processing enzymes is coordinately regulated and can be co-induced or coinhibited by regulation of cell surface receptors.

In the last two years, the Unit investigated the second biosynthetic step -- the mechanism of induction of POMC biosynthesis primarily using the POMC-containing cells of the intermediate lobe (IL) of the pituitary as a model. It was found that there are temporal differences in the way a neuroendocrine system regulates peptide biosynthesis. After long-term stimulation or inhibition, the secretory cells of the intermediate lobe proliferate or die in response to the requirement for POMC secretion. After a moderate length of stimulation or inhibition, inactive cells are recruited or active cells turned off. After acute stimulation, the biosynthesis of POMC is induced by increasing the intracellular content of POMC mRNA. The increase in POMC mRNA could be due to either a decrease in degradation rate of POMC or an increase in the POMC mRNA transcription rate. In FY 87 a tissue culture model of IL cells was developed to study the regulation of POMC mRNA and IL cell proliferation rate by dopamine, GABA, and serotonergic transmitter systems.

The studies regarding post-translational processing of POMC were continued in FY 87 with a study showing that dopaminergic agents selectively alter the post-translational processing of β -endorphin in the rat IL. In FY 87 studies on the regulation of β -endorphin processing in brain were initiated. It was found that chronic haloperidol treatment and ovarian steroids effect this processing.

In FY 86, a number of phosphoproteins associated with induction of POMC transcription and POMC peptide secretion were identified. In FY 87 these proteins were further characterized. In FY 87 we also found that the immune system may interact with the endocrine system as thymosin was found to regulate the secretion and biosynthesis of POMC peptides. Whether the source of the thymosin is actually the immune system is unclear, however, as the Unit has previously demonstrated that thymosin is present in the central nervous system.

2. Regulation of neuropeptide Y (NPY) biosynthesis.

In FY 84-86 the presence of neuropeptide Y (NPY) in the CNS was established by the Unit. An antibody was produced and characterized and used to map the distribution of NPY by immunohistochemistry and radioimmunoassay. In FY 87 NPY mRNA was first detected in the brain by in situ hybridization, establishing that NPY transcription occurs. In FY 86-87 a study of the NPY receptor was also accomplished. This distribution is quite similar to that seen in FY 84-85 for NPY containing nerve terminals, indicating that neuronally released NPY can react with these receptor populations.

This project involves investigation of the role of specific neurotransmitters in regulating neuronal activity in extrapyramidal systems. Current focus is on the nigrostriatal dopamine system which plays an important role in information processing in the basal ganglia. The function of these tonically active catecholamine neurons appears to involve regulation of gain and modulation of the effectiveness with which other neurotransmitter systems transmit information. These cells do not appear involved with transmission of specific phasic messages. Dysfunction of this neuronal system has been implicated in the etiology of many neurological diseases, including Parkinson's disease, tardive dyskinesia, schizophrenia and Huntington's Chorea.

In the past year, we have continued using neurophysiological techniques to explore the mechanisms underlying dopamine's critical role in the basal ganglia and the potential for modulating dopamine function with drugs. The focus of our studies has been an examination of three current problems relating to the different dopamine receptor subtypes:

1) What is the role of the D-1 dopamine receptor in basal ganglia function?

For many years, it has been assumed that the dopamine receptor responsible for mediating the classic effects of dopamine agonists on behavior was the D-2 dopamine receptor. The functional role of the D-1 receptor has been unclear. Previous studies in this section have lead to a fundamentally new and exciting concept about the relative roles of the two receptor subtypes: they indicate that D-1 and D-2 receptors interact synergistically to affect the activity of the striatal output cells and demonstrate the apparent necessity for both receptors to be simultaneously stimulated for the induction of processes previously thought independently mediated by the D-2 receptor. Additional studies have explored the cellular location of the receptors involved in mediating the change in basal ganglia output induced by the D-1 and D-2 agonists and focused on the possible modulatory role of the D-1 receptor. The results suggests that the interaction between the two receptor subtypes that accounts for the synergistic effect of D-1 and D-2 agonists on rat behavior and on the activity of cells in the globus pallidus and substantia nigra pars reticulata cannot be localized to globus pallidus or substantia nigra pars reticulata; lesion studies suggest that at least one site of action of the dopamine agonists with regard to induction of changes in the activity of the pallidal and nigral cells is the striatum. However, it has not been ruled out that the D-1 receptors on the striatonigral terminals may affect the function of the striatal output cells; iontophoretic studies suggest stimulation of these receptors may modulate the release of transmitter from the striatonigral neurons. Additional studies on the effects of D-1 receptor stimulation on the activity of dopamine cells have indicated that whereas processes mediated by D-1 and D-2 receptors located postsynaptic to dopamine neurons interact synergistically to affect basal ganglia neuronal output, processes elicited by D-1 receptors postsynaptic to the dopamine neurons and D-2 receptors on the dopamine neurons appear to be independently involved in dopamine agonist mediated regulation of dopaminergic activity.

- 2) Are the dopamine autoreceptors on the dopamine cell bodies and terminals different from the postsynaptic D-2 receptors?

Recently, much interest has focused on the question of whether the dopamine autoreceptors may constitute a distinct subset of receptors which could be selectively stimulated by a highly specific agonist. A drug selective for the dopamine autoreceptors might have some therapeutic advantages in the treatment of disorders like tardive dyskinesia, schizophrenia and Parkinsonism. In fact, several drugs have been shown to be more effective agonists at dopamine autoreceptors than at dopamine receptors postsynaptic to dopamine neurons both in behavioral studies and by our neurophysiological criteria. One hypothesis which has been advanced to explain this phenomena suggests that drugs with limited intrinsic efficacy will be less effective at postsynaptic dopamine receptors than at dopamine autoreceptors because there is a relative lack of spare receptors at postsynaptic sites. We have used EEDQ, a compound which irreversibly binds to dopamine (and norepinephrine) receptors, to investigate this spare receptor hypothesis. We found that reduction of the number of spare dopamine receptors by EEDQ treatment unmasks differences between the intrinsic activities of the relatively full agonist, apomorphine, and the putative dopamine autoreceptor agonists, BHT 920 and EMD 38362 at dopamine autoreceptors. Results also indicate that there are fewer spare dopamine receptors at postsynaptic sites influencing globus pallidus cell activity than at autoreceptor sites regulating dopamine cell activity. These studies provide neurophysiological evidence to support the hypothesis that the apparent selectivity of some dopamine agonists for dopamine autoreceptors is due to critical differences in the number of spare receptors at pre- and post-synaptic dopamine receptor sites. Thus, drugs like BHT 920, EMD 38362 and (-)-3PPP with weaker intrinsic activities appear to be selective for dopamine autoreceptors because there is a sufficient number of autoreceptors for them to elicit a full autoreceptor-associated response, but there is insufficient number of postsynaptic dopamine receptors for induction of a full postsynaptic response. The partial agonist/antagonist nature of these drugs is therefore more evident at postsynaptic sites.

- 3) How does denervation alter the consequences of dopamine receptor stimulation and affect the relative roles of the two receptor subtypes in the basal ganglia?

Unilateral 6-hydroxydopamine-induced lesion of the nigrostriatal dopamine pathway in the rat provides an animal model of Parkinsonism useful for investigations of drug effects and the nature of the changes induced by loss of dopamine neurons. Our Section has utilized extracellular single unit recording techniques to further examine the consequences of unilateral 6-hydroxydopamine-induced lesion of the nigrostriatal dopamine pathway in the globus pallidus and substantia nigra pars reticulata.

These studies show that removing the tonic inhibitory dopaminergic input to the basal ganglia does not cause significant changes in the firing rate of globus pallidus cells or render more globus pallidus cells inactive, but instead results in globus pallidus cell firing pattern changes. This would suggest that striatal neurons influenced by tonically active dopamine cells regulate

mechanisms controlling the temporal ordering of spontaneous discharges of globus pallidus cells. Recently, other researchers have reported similar findings with regard to the effects of 6-hydroxydopamine-induced lesion of the substantia nigra dopamine neurons on the activity of the substantia nigra pars reticulata neurons.

We have further explored the question of what denervation does to the relative roles of the D-1 and D-2 dopamine receptor subtypes in regulating extrapyramidal function in these animals. Behavioral studies have indicated that in the animal with supersensitive dopamine receptors, unlike the normal animal, D-1 receptor stimulation induces changes comparable to those exerted by the mixed agonists and by D-2 agonists. We have found that D-1 agonists induce relatively profound responses in the substantia nigra pars reticulata and globus pallidus in the supersensitive rats; D-2 effects are also potentiated in the globus pallidus but not in the substantia nigra pars reticulata. Our observations suggest that the similar behavioral effects induced by the D-1 and D-2 agonists in rats with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal pathway are not mediated via the substantia nigra pars reticulata since the two types of agonists are differentially effective at inducing neurophysiological changes at this site. Further studies comparing the effects of dopamine agonists in the 6-hydroxydopamine lesioned rats with effects in animals with more acute depletions of dopamine suggest that the lesion-induced changes are due to time-dependent alterations in processes affected by dopamine requiring more than five days to develop, or due to loss of something associated with the dopamine terminal other than dopamine. Neither AMPT pretreatment nor a 5 day reserpine treatment induced similar changes. Finally, present results support the findings of synergistic interactions between the receptor subtypes in the rats with supersensitive dopamine receptors; in these rats, as well as in normal animals, the two dopamine receptor agonists exert synergistic effects on substantia nigra pars reticulata cell activity. These studies suggest drugs which stimulate both receptor subtypes would be more efficacious in treatment of Parkinsonism than drugs selective for just one subtype.

Pharmacology Section

Extrapyramidal Disorders

Studies of extrapyramidal disease remain largely focused on the pathogenesis and treatment of the motor response fluctuations occurring in parkinsonian patients who have been chronically treated with levodopa. An evaluation of 27 individuals showed that the efficacy half-time for levodopa was substantially shorter in patients with wearing-off and especially in those with on-off phenomena compared to those with a stable response to levodopa or those never before treated with the dopamine precursor. In view of our inability to find any peripheral pharmacokinetic explanation for the development of motor fluctuations, the observed differences in efficacy half-time suggest involvement of central pharmacokinetic or pharmacodynamic mechanisms. The rapid clinical deterioration occurring in fluctuating responders presumably reflects an increased sensitivity to altered plasma and thus presumably cerebral levodopa levels. With advanced disease, most cerebral dopamine synthesized from

exogenous levodopa presumably originates in cells unable to store and release the transmitter amine under physiologic conditions. Dopamine levels at postsynaptic receptor sites could thus be expected to rapidly reflect the shifts in cerebral levodopa availability that attend the periodic administration of the dopamine precursor. Wearing-off phenomenon might arise simply as a consequence of the marked loss of dopaminergic neurons, resulting in a diminished ability to buffer treatment related fluxes in cerebral levodopa levels. The pathophysiology of the on-off phenomenon, on the other hand, may relate to postsynaptic as well as presynaptic factors since it is not completely eliminated by the acute stabilization of plasma levodopa levels. Ongoing clinical studies are now evaluating the possibility that postsynaptic changes, especially at the level of the dopamine receptor, may be an additional requirement for the appearance of on-off phenomenon.

There is reason to believe that an accumulation of certain levodopa metabolites might contribute to the pathogenesis of wearing-off or on-off phenomenon. To assess this possibility, 21 patients with idiopathic Parkinson's disease who manifested various response patterns to levodopa therapy were studied. Plasma OMD levels during chronic oral levodopa therapy or during steady state intravenous levodopa administration averaged about twice those of plasma levodopa. Plasma OMD concentrations correlated closely with plasma levodopa levels as well as with the dose of orally or intravenously administered levodopa. But plasma OMD levels during chronic oral levodopa therapy were far more stable than plasma levodopa levels and the coefficient of variance for plasma OMD failed to correlate with parkinsonian score variance. Furthermore, the ability of intravenously administered levodopa to diminish motor fluctuations correlated closely with a reduction in plasma levodopa oscillations, but not with any effect on plasma OMD variability. While our results do not exclude a central effect of OMD on the stability of motor responses to levodopa, they provide no support for the hypothesis that plasma OMD accumulations in parkinsonian patients receiving chronic levodopa therapy relate to the pathogenesis of either wearing-off or on-off responses.

D-1 and D-2 dopamine receptor mechanisms continue to be evaluated in relation to the control of motor function. Although D-2 receptors have generally been thought to mediate most dopaminergic influences, our earlier results in lesioned rats suggested that functional linkages between D-1 and D-2 receptors underlie certain of the behavioral effects of classical dopamine agonists that stimulate both receptor subtypes. Studies carried out in intact rats now provide additional support for this concept. The D-2 agonist LY 171555 augmented locomotor and grooming activity, while the D-1 agonist SKF 38393 increased only "intensive grooming." The full range of motor behaviors characteristically produced in nonlesioned animals by nonselective dopamine agonists occurred only with concurrent treatment with both agonists. Moreover, the behavioral responses to either SKF 38393 or LY 171555 given alone were inhibited in animals depleted of endogenous dopamine, but could be restored by the simultaneous administration of both the D-1 and D-2 agonists. These results suggest that in the normal animal unconditioned behaviors classically associated with the postsynaptic activity of dopamine agonists may require concurrent activation of both D-1 and D-2 receptors. Results of a collaborative study with the Physiologic Neuropharmacology Section, showing the effects of SKF 38393 and

LY 171555 on firing rates of rat globus pallidus cells mediating the neuronal output of the basal ganglia, support this view.

The clinical implications of the foregoing laboratory findings are also being explored. Oral administration of SKF 38393, alone or in combination with suboptimal rates of intravenously infused levodopa to parkinsonian patients, had no consistent effect on motor function. SKF 38393 given together with levodopa infused at supraoptimal rates also failed to modify parkinsonian signs but tended to diminish levodopa-induced dyskinesias. The scant clinical response to D-1 receptor agonist stimulation could not be explained by pharmacokinetic considerations: doses used were in the range found sufficient to modify rodent motor behavior; patient plasma-spinal fluid partition results suggested adequate drug penetration into the central nervous system; and patient spinal fluid drug levels were compatible with brain levels in rats receiving pharmacologically active doses of SKF 38393. The pathophysiology and pharmacology of Parkinson's disease may relate exclusively to the D-2 subclass of dopamine receptors, although an altered sensitivity of the D-1 receptor system might preclude any clinically significant effects from occurring with a selective D-1 agonist.

Clinical studies have continued to seek practical means for controlling motor fluctuations in parkinsonian patients. Although we have previously shown that continuous intravenous levodopa administration is effective in the short-term management of this complication, its use is relatively inconvenient due to the drug's low solubility and poor stability. Levodopa methyl ester (LDME), which can be readily hydrolyzed to levodopa by plasma esterases, is far more soluble and thus might serve as a suitable alternative. A controlled clinical trial of this hypothesis revealed that plasma levodopa variance was substantially less with intravenous LDME than with oral levodopa. Similarly, both average parkinsonian severity and variance scores decreased significantly on LDME. There was no clinical or laboratory evidence of LDME toxicity. Although the nature of this response closely resembled that which we have previously described with intravenous levodopa, LDME was substantially more convenient to administer: the volume of infusate was reduced by 97 percent thus enabling use of small wearable pumps.

Controlled release levodopa preparations provide another potential approach to the long-term management of motor fluctuations. Improved formulations have recently been developed including CR III in which levodopa and carbidopa are imbedded in a nonfragmentable polymer matrix. CR III administration to 17 parkinsonian patients produced reasonably stable plasma levodopa levels for at least 4 hours. Plasma variance was substantially less with CR III given every 4 hours than with standard levodopa given every 2 hours. Both parkinsonian severity and variance improved significantly with CR III. Nevertheless, CR III was poorly accepted by many patients without a small supplement of levodopa to offset the loss of the antiparkinsonian surge associated with standard levodopa ingestion. Advantages of CR III over levodopa included the convenience of less frequent dosing, improved sleep, and diminished early morning dystonia. Results with both LDME and CR III thus provide additional support for our earlier suggestion that pharmaceutical procedures that provide stable plasma levodopa levels will tend to produce stable motor responses in parkinsonian patients.

Studies of dementing disorders continue to focus on the development of an effective pharmacotherapy for Alzheimer's disease. A newly initiated approach involves the application of molecular pharmacologic techniques to evaluate potential pathogenetic mechanisms and test definitive pharmaceutical interventions. A second approach involves the use of classical transmitter pharmacology to develop drugs that might afford symptomatic relief. Implementation of this latter strategy continues to make use of functional imaging data to direct biochemical studies aimed at the identification of critical transmitter abnormalities in cerebral post mortem tissues. Since conclusions deriving from our initial PET-FDG investigations of Alzheimer's disease were based on results from patients who fulfilled the broad DSM-III criteria for primary degenerative dementia, they might have obscured the existence of pathogenetically distinct subgroups. During the past year a pilot study was completed which sought to explore this possibility by comparing PET-FDG scan patterns from demented patients who manifested predominant signs of language and/or visuospatial dysfunction with those who evidenced mainly disturbances in personality and social behavior. Five individuals selected because of preponderant "posterior" dementia, with disproportionate aphasic, apractic, or agnosic disturbances, had their most consistent abnormalities in the posterior parietal and temporal areas; in addition, two of these individuals had relatively mild areas of hypometabolism in the anterior frontal cortex. In contrast, five patients studied because of predominant signs of "anterior" dementia, with clinical changes mainly in personality and social behavior, consistently evidenced their most profound metabolic abnormality in the anterior frontal cortex. This frontal area was approximately the same as that having relatively mild hypometabolism in some patients with posterior dementia. Similarly, two of the anterior dementia patients also had relatively mild yet statistically significant abnormalities in the posterior temporal-parietal cortical areas which closely approximated those primarily involved in patients with posterior dementia. In addition, PET-FDG scans from three individuals with primary degenerative dementia, who relatively early in the course of their illness manifested approximately equal signs of both anterior and posterior dementia, showed roughly similar involvement of both the anterior frontal and posterior parietal-temporal cortices. Global measures of intellectual performance showed no significant differences between the patient groups; tests of visuospatial function were somewhat more abnormal in those with posterior dementia, while tests of concept formation and similarities tended to be more abnormal in those with anterior dementia.

The foregoing preliminary results suggest a characteristic pattern of cortical dysfunction occurs in patients with primary degenerative dementia. Most individuals referred for neurologic care who meet current diagnostic criteria for this disorder primarily evidence deficits in verbal and visuospatial cognition and receive the clinical diagnosis of Alzheimer's disease; a much smaller number present mainly with personality changes and disturbances in social behavior. The dichotomization of patients with primary degenerative dementia into anterior and posterior subtypes may be justified by the possibility that it will assist in the identification of distinct pathophysiologies and aid in the development of specific therapies. On the

other hand, we found that in both the anterior and posterior groups, maximal glucose hypometabolism can involve approximately the same cortical areas, albeit to different degrees. Moreover, both clinical and PET-FDG scan distinctions are most obvious at early stages of illness; the two groups apparently tend to merge as the disorder advances. Finally, bridging cases can be found, who early on combine in roughly equal proportions the profile of cognitive dysfunction and the pattern of cortical hypometabolism of both subgroups. These observations suggest that anterior and posterior subgroups may represent polar examples of a continuous spectrum of disease.

Continuing attempts to identify crucial transmitter abnormalities in Alzheimer's disease focused on corticotropin releasing factor (CRF) during the past year. Recent reports of profound reductions in cortical CRF levels in Alzheimer patients prompted a study of CRF-immunoreactivity in cerebrospinal fluid. A significant reduction in apparent spinal fluid levels of the neuropeptide were found in the 16 patients studied to date. No correlation could be found between CRF-I levels and age, symptom duration, or age at symptom onset. While mean CRF-I levels tended to increase over successive CSF aliquots collected from both Alzheimer patients and control subjects, this trend was less apparent in the patient group. The lack of a definite rostrocaudal gradient for CRF-I in spinal fluid probably reflects the wide distribution of the neuropeptide throughout the central nervous system, including spinal cord. The magnitude of the CRF difference between patients and controls is much more striking in brain tissue than in spinal fluid. Presumably, CRF in subcortical regions, especially hypothalamus and spinal cord, gains access to the CSF, thus minimizing changes in the lumbar sack produced by changes in the cerebral cortex. Although the overlap of individual spinal fluid CRF-I levels between Alzheimer patients and control subjects casts doubt on the diagnostic potential of this determination, the possibility that the assay of this peptide in combination with other possible CSF markers of Alzheimer's disease might prove useful is currently being explored. Unlike the strong relation between the degree of cognitive dysfunction and the degree of cortical acetylcholine or somatostatin deficiency, however, no such relation was found with CRF in lumbar spinal fluid. This might reflect the diluting out of spinal fluid CRF changes due to cortical abnormalities by normal rates of CRF production at other sites along the neuraxis. On the other hand, the reported distribution of cortical CRF deficits does not appear to correlate well with the predominately parietal association cortex dysfunction revealed by our PET-FDG studies. Conceivably, CRF system abnormalities play a secondary rather than a primary role in the pathogenesis of Alzheimer dementia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02263-11 ET
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Pharmacological Studies of Dopamine Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Sibley, Ph.D., Head, Molecular Pharmacology Unit, ETB, NINCDS T. Agui, Visiting Fellow, T. Yamamoto, Visiting Fellow; Y. Hatada, Visiting Fellow, M. Munemura, Visiting Scientist, ETB, NINCDS		
COOPERATING UNITS (if any) None		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Biochemical Neuropharmacology Section/Molecular Pharmacology Unit		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 4.2	PROFESSIONAL: 3.7	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) D-1 dopamine receptors in the substantia nigra and retina were characterized with ligand binding and autoradiographic techniques using the newly developed ligand using [¹²⁵ I] SCH 23982. The role of cAMP, ATP and calcium in regulating α-MSH release from the rat neurointermediate pituitary gland was investigated. The data indicated that cAMP and ATP directly modulate α-MSH secretion rather than modulating calcium concentrations. A thermodynamic analysis of agonist and antagonist ligand interactions with D-2 dopamine receptors was performed. Several agonist and antagonist ligands exhibited anomalous binding properties. Desensitization of the D-2 dopamine receptor in the neurointermediate pituitary gland was demonstrated. The data indicated that the D-2 receptor is capable of undergoing direct functional modification. Solubilization procedures for both D-1 and D-2 dopamine receptors were developed using bovine and canine striatal tissue. Purification techniques and procedures for the large scale purification of D-1 and D-2 dopamine receptors were initiated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02578-05 ET
PERIOD COVERED October 1, 1986 through September 30, 1987.		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology and Cellular Biology of Peptidergic Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bibbie M. Chronwall, Ph.D., Acting Head, Neuroendocrinology Unit, ETB, DIR, NINCDS T.N. Chase, P.C. Contreras, J.M. Farah, W.R. Millington, D.R. Gehlert, M.M. Mouradian, T.L. O'Donohue, ETB, DIR, NINCDS		
COOPERATING UNITS (if any) NICMS PRAT, Uniformed Services Univ., Searle Research and Development, Univ. California at Davis, Univ. Florida in Gainesville		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Neuroendocrinology Unit		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 5.6	PROFESSIONAL: 4.1	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this project is to develop an understanding of the basic regulatory mechanisms in neurons and endocrine cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic neuronal and endocrine systems. Two studies are ongoing. The first examines the regulation of biosynthesis of proopiomelanocortin (POMC) in the neuronal and endocrine opioid system which secrete ACTH, α-MSH, and δ-endorphin- peptides all derived from a common prohormone, POMC. Our investigations indicate that regulation of POMC gene expression occurs primarily at the transcriptional level. The expression of POMC and processing enzyme genes appear to be regulated by cAMP-protein kinase A and diacylglycerol-protein kinase C mediated mechanisms. We have identified a number of putative third messenger phosphoproteins which may link cell surface receptors to transcriptional mechanisms in the nucleus. The second study is focused on biosynthesis of neuropeptide Y (NPY) in brain and a tissue culture model consisting of the pheochromocytoma tumor cell line PC-12. NPY has been established as a neurotransmitter candidate in the central nervous system. It is widely distributed and present in high concentrations, is active as a vasoconstrictor and modulates endocrine, appetite and cognitive functions. NPY transcription occurs in the brain as well as in the PC-12 cell line. The NPY binding sites have been mapped in the brain and a characterization of the molecular properties of the receptor is being accomplished.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02139-13 ET
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Judith R. Walters, Ph.D., Chief, Physiological Neuropharmacology Section, Experimental Therapeutics Branch, NINCDS		
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Joanne Carlson, Ph.D., Dept. Psychiatry, School of Medicine, University of California San Diego		
COOPERATING UNITS (if any) Pharmacology Section, Experimental Therapeutics Branch, NINCDS		
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INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 5.1	PROFESSIONAL: 4.1	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 1) <u>The D-1 dopamine receptor in basal ganglia function.</u> Previous studies have indicated that D-1 and D-2 receptors interact synergistically to affect the activity of the striatal output cells. Moreover, it appears that both receptors need to be simultaneously stimulated for the induction of many processes previously thought independently mediated by the D-2 receptor. Current results suggest that the site of interaction between the two receptor subtypes cannot be localized to the globus pallidus or substantia nigra pars reticulata; lesion studies suggest that at least one site of action of the dopamine agonists with regard to induction of changes in the activity of the pallidal and nigral cells is the striatum. However, D-1 receptors on the striatonigral terminals may affect the function of the striatal output cells; iontophoretic studies suggest stimulation of these receptors may modulate the release of transmitter from the striatonigral neurons. 2) <u>Selective modulation of dopamine autoreceptor function.</u> Certain drugs whose therapeutic potential is a matter of current interest appear selective for dopamine autoreceptors. Does this indicate that dopamine autoreceptors are different from postsynaptic dopamine receptors? Our studies suggest otherwise; they support the hypothesis that the apparent selectivity of some dopamine agonists for dopamine autoreceptors is due to differences in the number of spare receptors at pre- and post-synaptic dopamine receptor sites. Thus, drugs with weaker intrinsic activities appear to be selective for dopamine autoreceptors because there is a sufficient number of autoreceptors for them to elicit a full autoreceptor-associated response, but there is insufficient number of postsynaptic dopamine receptors for induction of a full postsynaptic response. The partial agonist/antagonist nature of these drugs is therefore more evident at postsynaptic sites. 3) <u>Consequences of dopamine receptor denervation.</u> Current studies indicate that striatal neurons influenced by tonically active dopamine cells regulate mechanisms controlling the temporal ordering of spontaneous discharges of globus pallidus cells.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02265-11 ET
PERIOD COVERED October 1, 1986 through September 1, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology, Biochemistry and Physiology of Central Neurotransmitters		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Thomas N. Chase, M.D., Chief, Pharmacology Section, Experimental Therapeutics Branch, NINCDS P. Barone, H. Burrows, T. Engber, G. Fabbri, J. Juncos, M. Mouradian, S. Parashos, G. Schlegel, ETB, DIR, NINCDS		
COOPERATING UNITS (if any) Department of Psychiatry, University of Maryland; Tissue Research Center, Harvard University; Department of Neurology, University of California Los Angeles.		
LAB/BRANCH Experimental Therapeutics Branch, DIR NINCDS		
SECTION Pharmacology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 10	PROFESSIONAL: 8	OTHER: 2
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this project is to develop improved pharmacotherapies for selected neurodegenerative disorders, especially Parkinson's disease and Alzheimer's disease. Studies of Parkinson's disease suggest that motor fluctuations of the wearing-off type primarily reflect dopamine cell loss, with a resultant inability to buffer treatment related fluxes in cerebral levodopa; on-off phenomenon reflects additional postsynaptic alterations. Circulating 3-O-methyldopa contributes to neither state. Despite preclinical observations indicating D-1 as well as D-2 receptor stimulation may be required for a complete dopamine agonist response, an analogous role for D-1 receptors has not been found in the treatment of parkinsonian patients. Both the infusion of levodopa methylester and the ingestion of a controlled release levodopa preparation proved to be relatively practical approaches to the alleviation of motor fluctuations. Continuing application of the positron emission tomographic-fluorodeoxyglucose technique has distinguished two subgroups of primary degenerative dementia: the most common type presents mainly with verbal and/or visuospatial deficits and evidences cortical hypofunction primarily in the parietal-temporal area; a smaller group presents mainly with alterations in personality and/or social behavior and evidences cortical hypofunction primarily in the frontal area. In view of overlapping involvement, both groups may represent polar examples of a continuous disease spectrum. Biochemical studies revealed diminished cerebrospinal fluid levels of corticotropin releasing factor. Since spinal fluid concentrations did not correlate with neuropsychological function, the CRF system deficits may contribute relatively little to the pathogenesis of Alzheimer dementia.		

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ANNUAL REPORT

October 1, 1986 through September 30, 1987

Infectious Diseases Branch

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1986 through September 30, 1987
Infectious Diseases Branch, DIR
National Institute of Neurological
and Communicative Disorders and Stroke

John Louis Sever, M.D., Ph.D., Chief

I. RESPONSIBILITY OF THE BRANCH

The responsibility of the Infectious Diseases Branch is to carry out coordinated research programs concerned with infections which damage the human nervous system. The Branch is divided into three Sections: 1) Immunochemistry and Clinical Investigations (ICI); 2) Experimental Pathology (EP); and 3) Neurovirology and Molecular Virology (NMV). These Sections utilize the techniques of clinical investigations including human volunteers and clinical trials, experimental pathology with small laboratory animals and nonhuman primates, virology, immunology, recombinant DNA technology and gene expression, tissue culture, and electron microscopy.

II. PROGRAM SEGMENTS

The program segments are: a) perinatal; b) acute; and c) chronic. In each segment we are concerned with: 1) etiology and diagnosis; 2) mechanisms of pathogenesis; 3) treatment; and 4) prevention.

III. RESEARCH AREAS

The present research areas in the program segments include:

A. Perinatal

Investigate methods for the early diagnosis of infections which damage the CNS and study mechanism of pathogenesis and prevention. Current studies include pathogenesis of cytomegalovirus infections; cofactors in AIDS; and microcephaly in congenital AIDS.

B. Acute

Investigate agents which may be responsible for acute neurological diseases. Current studies relate to the pathogenesis of varicella-zoster infections which lead to shingles.

C. Chronic

Study chronic neurological diseases using combined approaches for possible infectious etiologies and mechanisms of pathogenesis. Whenever possible, explore methods for early diagnosis, treatment and prevention. Current studies include progressive multifocal leukoencephalopathy, SAIDS and AIDS.

IV. SECTION ACTIVITIES

A. Section On Immunochemistry and Clinical Investigations (ICI)

1. Perinatal

a. Early Diagnosis

A new method was developed for the rapid typing of herpes viruses using a capture system with polyclonal antibody and a detection system with biotinylated monoclonal antibody. This permits the rapid and specific typing of isolates.

b. Virus Model To Study Myelinogenesis

We established sheep glial cultures and studied the effect of BD virus on enhanced cultures of oligodendrocytes, astrocytes, precursor cells, dorsal root ganglion cells, and Schwann cells. The virus was not restricted in vitro and grew well in all cell types and ages of cells.

c. Cytomegalovirus

We studied the immune response of pregnant rhesus monkeys following intrauterine inoculation with rhesus cytomegalovirus. Animals injected in utero showed a much more extensive antibody response than did animals inoculated at birth. Other immunological functions are not different for the two types of inoculation.

An investigation of children with congenital CMV infection also showed more extensive and varied immune response than did children infected after birth.

d. AIDS

We are investigating the susceptibility of newborn-cord lymphocytes to the HIV virus compared to adult lymphocytes. We are also testing samples of blood, amniotic fluid and tissues from infants of women who have antibody to the HIV virus.

We are studying biopsy and autopsy samples from patients with various forms of "Neurological AIDS" to determine the pathogenesis of the infection.

e. Collaborative Study

We have tested serum specimens for HIV antibody from 75 pregnant women who were IV drug abusers and participated in the Collaborative Perinatal Study 1960-1966. Two of these women had HIV antibody positive specimens. Further study showed these reactions to be due to HLA antibody in the sera reacting with HLA antigens in the virus preparation. This is an important source of false positive HIV reactions.

We studied serum specimens from pregnant women who delivered children with microcephaly and matched controls for antibody to a putative human pestivirus. Two of the women who delivered children with microcephaly had neutralizing antibody to two pestiviruses. None of the controls had antibody. This suggests the possible role of pestiviruses in human dysmyelination and microcephaly.

2. Acute

a. Clinical Studies - Reye's Syndrome

Children who survived Reye's Syndrome and other members of their families were entered in a study of aspirin metabolism. Aspirin has been epidemiologically associated with Reye's Syndrome. The children had high levels of varicella and influenza antibodies. Metabolism of aspirin was not unusual for the Reye's Syndrome survivors compared to siblings and parents.

3. Chronic

a. AIDS

Antibodies to a variety of agents were studied in homosexual men from Washington, D.C., and New York City area and compared to control blood donors. Antibodies to HSV-1, HSV-2, CMV and EBV were all elevated in the homosexual men. These studies show exposure to many viruses which could serve as "co-factors" in the development of AIDS when individuals are exposed to the HIV virus.

In vitro simultaneous infection of lymphocytes with CMV and HIV showed increased replication of HIV in the mixed infection compared to cells infected with HIV alone. This demonstrates the cofactor effect of CMV on HIV replication in human lymphocytes.

We have initiated a clinical protocol to evaluate AZT for the treatment of AIDS patients with neurological symptoms. Patients with dementia, peripheral neuropathy, and polymyositis are being treated with AZT and evaluated in detail.

B. Section On Experimental Pathology (EP)

1. Perinatal

a. Rhesus Cytomegalovirus (RCMV)

We are participating in studies of the immune response of rhesus monkeys following infection with RCMV in utero and as newborns. (With ICI Section).

2. Acute

a. Simian Varicella

We are participating in investigations of new strains of Simian varicella which produce acute, severe infection in some monkey specimens and asymptomatic or mild symptoms in others. These symptoms permit the study of latency and reactivation of varicella. (With NV Section).

3. Chronic

a. AIDS and SAIDS

We are investigating various strains of HIV and STLV III in non-human primates as models of neurovirulent AIDS in experimental models. (With NV Section).

b. JC - PML

Neuro-oncogenic studies are being continued in nonhuman primates with variants of the JC virus as part of our studies of PML. (With NV Section).

C. Section On Neurovirology & Molecular Virology (NMV)

1. Perinatal

AIDS

We are studying samples of amniotic fluid cells and peripheral blood from pregnant women for isolation of HIV virus. This information is correlated with clinical and serological data for the pregnancies.

2. Acute

Varicella-Zoster

We are comparing new isolates of Simian varicella to identify new strains. One new strain has unusual biological characteristics. This strain is very virulent for patas and African green monkeys but not for rhesus. This permits the study of acute and latent varicella and factors involved in reactivation.

Comparison by electrophoresis and acrylamide gel shows the new isolate to be closely related to the human varicella virus.

3. Chronic

a. SAIDS

We have studied the SAIDS outbreak at NIH and developed procedures for detecting infected animals and controlling the spread of infection. We have demonstrated that antibody studies cannot be used to identify infected animals. The isolates have been shown to be SRV-2.

We are investigating new isolates of STLV-III from African green monkeys as part of our effort to establish a neurovirulent model of AIDS. The new isolates infect T-4 cells and by EM are very similar to the human HIV virus. Growth of the STLV-III virus can be blocked with antibody to T-4. Rhesus monkeys have been inoculated with various isolates of STLV-III (SIV_{AGM}) and some of the animals are showing disease.

b. JC Virus - PML

Studies on the molecular interaction of JC virus and human glial cells have revealed that viral early gene transcription is regulated by cell specific proteins. These proteins presumably bind to JCV DNA sequences in the non-coding or regulatory region of the viral genome. Viral DNA replication, on the other hand, is regulated by specific proteins that are found in a variety of primate cells not necessarily only in glial cells. The clinical studies of

JC virus induced progressive multifocal leukoencephalopathy (PML) have identified replicating viral DNA in biopsy tissues of cases in which PML was suspected from clinical examinations and histopathology of tissue specimens. A new strain of JCV has been identified from an owl monkey astrocytoma cell line, JCV 586. This virus produces an early gene product, T protein, which is recognized by monoclonal antibodies to the JV 40 T protein. The JCV 586 T protein also is able to complex with a host cellular nuclear oncogene product p53. These characteristics differ from the prototype JC virus. Therefore JCV 586 provides the opportunity to study altered viral gene functions.

V. FINDINGS

A Perinatal

1. Rapid typing of herpes group isolates can now be accomplished with the new capture-biotin monoclonal antibody system which was developed in our laboratory.
2. The BD virus was shown to grow well in all 5 cell types of brain tissue studied. This shows that localization of the infection in certain parts of the CNS in vivo is not a function of cell tropism.
3. Several women who had children with microcephaly had antibody to pestiviruses. This suggests the possible role of these viruses in human neurological disease.
4. Children and experimental animals infected with CMV in utero were shown to have markedly increased and broadened antibody responses. This suggest heightened infection in vitro and augmented immune response.
5. Several IV drug abusers who were pregnant in 1960-1966 had positive antibody tests for HIV. These reactions were due to cellular HLA responses and not to the virus. This is important to consider in perinatal AIDS studies.

B. Acute

1. Children who survived Reye's Syndrome did not have difficulty metabolizing aspirin when given varying doses of the drug. This demonstrates that the children have no functional defect in metabolism of this drug.
2. New strains of Simian varicella provides models of acute and subacute varicella. This is of great value in studies of latency and reactivation of varicella.

C. Chronic

1. AIDS/SAIDS

- a. Studies of homosexual men in Washington, D.C., and New York City area have shown evidence of multiple viral infections in these individuals. This demonstrates that a number of possible viral cofactors for AIDS are present in these men.
- b. In vitro tests showed that CMV acts as a cofactor and stimulates the growth of HIV in lymphocytes.
- c. Patients with neurological complications are showing some improvement when treated with AZT.
- d. Nonhuman primate studies with STLV-III show increased virulence of the virus on animal to animal passage.
- e. A SAIDS outbreak in animals at NIH has shown that antibody screening cannot be used to identify infected animals.
- f. Patient with AIDS may present polymyositis as the only complaint while others may develop this during the course of their disease.

2. JC - PML

- a. JCV DNA replication is controlled by species specific factors as well as the synthesis of its own non-structural T protein.
- b. Transcription of the JCV early gene is regulated in a cell specific manner which recognizes enhancer and promoter sequences in the regulatory sequences of its DNA.
- c. JCV 586 DNA isolated from a transplantable owl monkey astrocytoma contains a 19 bp deletion in the second tandem repeat of the viral enhancer sequence. This identifies the released virus as the Mad-4 strain.
- d. JCV 586 T protein binds the host cellular p53 protein, a nuclear oncogene product. The T protein also is recognized by SV 40 T protein monoclonal antibodies which bind to epitopes at both the amino terminal and carboxy terminal ends of the protein, indicating some structural homology between the JCV 586 and SV 40 T proteins.
- e. Owl monkey astrocytoma cells in culture which release JCV 586 comprise about 30% of the viable tumor cells, indicating that a persistent carrier culture has been established.
- f. JCV was isolated from a case study of progressive multifocal leukoencephalopathy. The viral DNA was cloned into a plasmid vector and partially sequenced. The isolated virus was identified as a Mad-4 strain based upon the DNA sequence information.

g. Brain biopsy specimens that were paraffin embedded were analyzed by in situ DNA: DNA hybridization for the presence of JCV DNA. All cases were positive which correlated with clinical findings and histopathology seen in the biopsy. Hybridization tests were able to definitely diagnose these cases of JCV induced PML.

3. Post Polio Neuromuscular Symptoms

- a. The pathogenesis of this syndrome appears to relate to the loss of the distal nerve terminals and lack of regeneration of groups but not due to loss of the whole motor neuron as is seen in the prototype of motor neuron disease - amyotrophic lateral sclerosis.
- b. Based on clinical, electrophysiological, muscle biopsy and histochemical criteria, the neuromuscular symptoms of the patients were specifically defined and categorized. This has been useful for the recognition of progression, management and design of experimental therapies.

CONTRACT NARRATIVE

Infectious Diseases Branch, DIR, NINCDS

Fiscal Year 1987

Microbiological Associates: (NO1-NS-7-2392)

Title: Development and Delivery of Antigen, Antisera, and Viral Diagnostic Reagents.

Contractor's Project Director: Dr. David A. Fuccillo

Current Funding: \$175,000.00

Objectives: This is a service contract to provide research reagents for studies of neurological diseases which may have infectious etiologies and special investigations of polyomaviruses, AIDS and simian AIDS (SAIDS).

Major Findings: Antigens to be used as diagnostic reagents have been produced for HTLV-I, herpes virus types I and II, cytomegalovirus, measles, rubella, varicella viruses and the parasite toxoplasma gondii. These antigens were used in studies on the etiology of neurological diseases. Evaluation of the antigens, conjugates and controls required to produce specific and sensitive enzyme-linked immunosorbent assays (ELISA) was performed. Reagents for the study of acquired immune deficiency syndrome (AIDS) as well as an AIDS-like disease in monkeys (SAIDS) caused by a group of simian retroviruses are being developed and tested. For the detection of antibody by indirect immunofluorescence the following antigens have been prepared: HTLV-I, HTLV-III, SRV-I and STLV-III. These reagents have been evaluated using sera from selected monkeys and human populations. Reagents to study rhesus monkey CMV and its relationship to SAIDS have been prepared. Antibody was purified and horseradish peroxidase conjugates were prepared for the study of Border Disease (BD) - a neurological disease of sheep. Reagents have been prepared for studies on the molecular genetics of the BK and JC virus.

Preparation and purification of plasmid DNAs have been produced for four recombinant DNA constructions. Two of these plasmid DNAs contain eucaryotic gene sequences which code for proteins expressed in human fetal brain; namely the p53 phosphoprotein involved in cell cycle and transformation and glial fibrillary acidic protein (GFAP), the structural intermediate filament of astroglial cells. Two other plasmids contain segments of the viral genome of the neurotropic human polyomavirus JCV. For each of these DNA constructions, large volumes of plasmid carrying host E. coli bacteria were grown and the plasmid DNA isolated. These plasmids were analyzed following purification from host DNA by restriction endonuclease mapping to assure that alteration had not taken place during plasmid growth.

Publications

Shekarchi, I.C. and Sever, J.L.: Enzyme immunoassay. In Clinical Virology Manual, S. Specter and G.J. Lancz (eds.), Elsevier, New York, 1986, 133-146.

Nerurkar, L.S., Biggar, R.J., Goedert, J.J., Wallen, W., Becker, P., West, F., Tzan, N., Traub, R., Lee, Y.J., Botelar, W., Fuccillo, D., Madden, D.L. and Sever, J.L.: Antiviral antibodies in the sera of homosexual men: correlation with their lifestyle and drug usage. J. Med. Virol., 21:123-135, 1987.

Fuccillo, D.A., Madden, D.L., Tzan, N. and Sever, J.L.: Difficulties associated with serological diagnosis of Toxoplasma gondii infections. Diagn. Clin. Immunol., 5:8-13, 1987.

Shekarchi, I.C., Fuccillo, D.A., Strouse, R. and Sever, J.L.: Capillary enzyme immunoassay for rapid detection of herpes simplex virus in clinical specimens. J. Clin. Microbiol., 25(2):320-322, 1987.

Fuccillo, D.A.: Congenital Varicella. In Teratogen Update: Environmentally Induced Birth Defects Risks. J.L. Sever and R.L. Brent (eds.), Alan R. Liss, Inc. New York, 1986.

Fuccillo, D.A. and Sever, J.L.: Monoclonal antibodies as reagents. In Concepts in Viral Pathogenesis II, A.L. Notkins and M.B.A. Oldstone (eds.). Springer-Verlag, New York, 1986, 324-320.

Fuccillo, D.A. and Sever, J.L.: Measles, mumps, and rubella. In Clinical Virology Manual, S. Specter and G.J. Lancz (eds.), Elsevier, New York, 1986, 437-449.

Ferranti, P., Fuccillo, D.A., Tzan, N.R., Madden, D.L., Mancuso, R. and Sever, J.L.: Measles specific immunoglobulins in SSPE patients. In Subacute Sclerosing Panencephalitis: A Reappraisal. F. Bergamini, C.A. Defanti and R. Ferrante (eds.). Elsevier Science Publishers B.V. (Biomedical Division), 1986, 337-342.

Sever, J.L., Madden, D.L., Fuccillo, D.A., Budzko, D., Ferrante, P., Dyken, R.R. and Dotson, P.M.: Immunological changes in patients with SSPE. In Subacute Sclerosing Panencephalitis: A Reappraisal. F. Bergamini, C.A. Defanti and R. Ferrante (eds.). Elsevier Science Publishers B.V. (Biomedical Division), 1986, 263-273.

South, M.A., Rodriguez, W., Fuccillo, D., Nerurkar, L., Yachie, A. and Sever, J.L.: Immunologic findings in a case of congenital CMV compared to infants with AIDS. In Viruses, Immunity, and Immunodeficiency, A. Szentivanyi and H. Friedman (eds.), Plenum Publishing Corporation, New York, 1986, 67-72.

South, M.A., Fuccillo, D.A. and Sever, J.L.: Cytomegalovirus infection and the immune system. In Viruses, Immunity, and Immunodeficiency, A. Szentivanyi and H. Friedman (eds.), Plenum Publishing Corporation, New York, 1986, 59-66

CONTRACT NARRATIVE

Infectious Diseases Branch, DIR, NINCDS

Fiscal Year 1987

SEMA, Inc.: (N01-NS-5-2377)

Title: Isolated Housing and Care of Animals Used in Studies of Infectious Diseases of the Central Nervous System.

Contractor's Project Director: Dr. Jere M. Phillips

Date Contract Initiated: 16 May 1987

Current Annual Level: \$173,293.00

Objectives: The contract provides isolated housing and care for laboratory rodents and a colony of nonhuman primates consisting of several genera. The animals are on experimental studies directed by written protocols. They require monitoring daily for clinical signs of disease. Biological specimens are collected as prescribed by protocols. The aims of the contract are to provide the facilities which permit animal studies that are judiciously planned to be humanely carried out. Animal studies carried out for the IDB, NINCDS are designed to investigate the etiology, pathogenesis, early diagnosis, treatment and prevention of both known and suspected infectious diseases of the nervous system. Studies with simian retroviruses are also conducted using the excellent containment facilities available under this contract.

Methods Employed: Animals are quarantined, conditioned and screened for preexisting antibodies to agents under investigation. Seronegative animals are inoculated by a variety of routes. The infected animals are then held in individual isolation units, monitored and tested as directed in written protocols.

Major Findings: This contract satisfactorily provides housing and care for most of the laboratory animals needed for research in the Infectious Diseases Branch. Animals are used in a number of studies of the infections of the central nervous system (CNS). Experimental animals which become sick are promptly identified and supportive therapy instituted. The investigators on the contract provide overall daily clinical care for the entire colony, with strict isolation procedures carried out at all times. The Contractor's Project Director makes modifications of studies when necessary to achieve the overall goals of the contract. The facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The accredited guidelines are designed to assure proper and humane care of all laboratory animals. These guidelines are strictly adhered to by facilities funded under this contract.

Publications: All publications from this contract are listed in the Annual Report of the Infectious Diseases Branch in each Section.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-00402-31-ID												
PERIOD COVERED October 1, 1986 through September 30, 1987														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Perinatal Infections Causing Damage to the Children in the CPP														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI John L. Sever, M.D., Ph.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 34%;">IDB, DIR, NINCDS</td> </tr> <tr> <td>Others: Jonas Ellenberg, Ph.D.</td> <td>Chief</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Nancy Madden</td> <td>Microbiologist</td> <td>IDB, DIR, NINCDS</td> </tr> <tr> <td>Bishara Freij</td> <td>Visiting Fellow</td> <td>IDB, DIR, NINCDS</td> </tr> </table>			PI John L. Sever, M.D., Ph.D.	Chief	IDB, DIR, NINCDS	Others: Jonas Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS	Nancy Madden	Microbiologist	IDB, DIR, NINCDS	Bishara Freij	Visiting Fellow	IDB, DIR, NINCDS
PI John L. Sever, M.D., Ph.D.	Chief	IDB, DIR, NINCDS												
Others: Jonas Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS												
Nancy Madden	Microbiologist	IDB, DIR, NINCDS												
Bishara Freij	Visiting Fellow	IDB, DIR, NINCDS												
COOPERATING UNITS (if any) BFSB, DIR, NINCDS														
LAB/BRANCH Infectious Diseases Branch														
SECTION Immunochemistry and Clinical Investigations														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:												
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this study is to determine insofar as possible the role of <u>perinatal infections</u> in the production of <u>fetal damage</u>. To accomplish this, clinical data and a large number of <u>serial serum specimens</u> were obtained from the <u>58,000 women</u> and their children in the <u>Collaborative Perinatal Project</u>. A number of reports and publications have come from the study. During this year papers have been published summarizing approaches used by the study and the <u>incidence of clinical infections</u> in the study population. Current efforts have been focused on completing the analysis and publication of the two remaining major studies from the project: 1) <u>Toxoplasmosis and Fetal Damage</u>, 2) <u>Papilloma Viruses And Fetal Damage</u>, and 3) <u>The Study Of The Pregnancies Of Abnormal Children And Matched Controls</u>. We are also <u>supplying clinical data and serum specimens</u> from the Collaborative Project for several other studies including: NICHD Study On <u>Maternal Diabetes</u>; NIAID Investigations Of <u>AIDS</u>; University of California, Berkeley studies of <u>cancer and thyroid disease</u>; and a study with the CDC on <u>fetal abnormalities</u> caused by <u>parvovirus infections</u>.														
12-IDB/DIR														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02532-05-ID

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of AIDS and SAIDS Neurological Findings and Etiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. J.L. Sever, M.D., Ph.D. Chief, IDB, DIR, NINCDS

Others: M. Gravell, Ph.D. Research Microbiologist IDB, DIR, NINCDS
W.T. London, D.V.M. Veterinary Director IDB, DIR, NINCDS
D.L. Madden, D.V.M., Ph.D. Veterinary Director IDB, DIR, NINCDS
M.C. Dalakas, M.D. Medical Officer IDB, DIR, NINCDS
G. Elder, M.D. Medical Staff Fellow IDB, DIR, NINCDS
L. Nerurkar, Ph.D. Special Expert IDB, DIR, NINCDS
M. Monzon, Ph.D. Visiting Scientist IDB, DIR, NINCDS
B. Freij, M.D. Visiting Fellow IDB, DIR, NINCDS

COOPERATING UNITS (if any)

Section of Neurovirology, IDB, DIR, NINCDS

Section of Experimental Pathology, IDB, DIR, NINCDS

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

11.6

PROFESSIONAL:

3.8

OTHER:

7.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Normal human peripheral blood mononuclear cells cultured in vitro can be doubly infected with human immunodeficiency virus (HIV) and cytomegalovirus (CMV). Doubly infected cells produce augmented titers of HIV. In contrast to CMV, neither herpes simplex virus, type 1 or 2, augmented HIV replication in doubly infected cultures of H9 cells.

The capacity of various T and B cell lines to support replication of HIV, simian immunodeficiency virus (SIV) and SAIDS retrovirus type 1 (SRV-1) was studied. We have found that the T-cell lines, CEM, Rex, Molt-3 and Peer, all of which contain both CD4 and CD8 surface markers, supported HIV replication. SIV replicated in only two of the T-cell lines, CEM and Molt-3. A T-cell line, HSB-2, which had neither CD4 or CD8 surface markers, did not support HIV replication. B-cells containing the EBV genome supported replication of HIV, but the titers of infectious virus produced were quite low. SRV-1 did not replicate in any of the T-cell lines studied, but replicated well in B-cell lines containing the EBV genome.

We have isolated a retrovirus with characteristics of SIV from three wild-caught, African green monkeys. Rhesus monkeys infected with these SIV isolates have developed an immunosuppressive disease similar to human AIDS.

An epizootic of simian acquired immunodeficiency syndrome (SAIDS), caused by a Mason-Pfizer-like type D retrovirus, has been detected in rhesus (M. mulatta) and cynomolgous monkeys (M. fascicularis) housed in the NIH colony. Neutralization tests have shown this Mason-Pfizer-like virus to be SAIDS retrovirus, type 2 (SRV-2). Procedures have been formulated to prevent further spread of this agent to susceptible animals.

13-IDB/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-01985-16-ID
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Perinatal and Neurological Diseases - Viral and Nonviral Antigens or Antibodies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI John L. Sever, M.D., Ph.D. Chief IDB, DIR, NINCDS Others: L. Nerurkar, Ph.D. Special Expert IDB, DIR, NINCDS S. Deshmane, Ph.D. Visiting Fellow IDB, DIR, NINCDS B. Freij, M.D. Visiting Fellow IDB, DIR, NINCDS M. Ceroni, M.D. Guest Researcher IDB, DIR, NINCDS		
COOPERATING UNITS (if any) Microbiological Associates, Inc. University Medical School, Milano, Italy University of South Alabama, Mobile, Alabama		
LAB/BRANCH Infectious Diseases Branch		
SECTION Immunochemistry and Clinical Investigations		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.75	OTHER: .75
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Healthy homosexual men between the ages of 21 and 65 years, from the Washington, DC (n=162), and New York City (n=89) areas, were studied for antibodies in the serum against cytomegalovirus (CMV), herpes simplex virus (HSV) types 1 and 2, and Epstein Barr virus (EBV) viral capsid antigen (VCA). CMV-specific antibodies were assayed by enzyme-linked immunosorbent assay (ELISA), anti- HSV-1 and HSV-2 antibodies were measured by indirect hemagglutination (IHA), and antibodies to EBV VCA were measured by the immunofluorescence assay. Antibodies to human T lymphotropic virus III (HTLV-III) were detected by ELISA and Western blot procedures. T lymphocytes were enumerated using OKT4 monoclonal antibody. Healthy male volunteer blood donors (n=90) matched for age range and race proportions were used as controls. The percentage of seropositive individuals in the homosexual group was higher (90-98%) for all the viruses tested than in the control group (47-87%). Comparisons of the geometric mean titers, expressed as reciprocal serum dilutions, of seropositive individuals in homosexual (H) vs control (C) group were as follows: CMV-IgG (ELISA) H = 1:794, C = 1:68; HSV-1 (IHA) H = 1:248, C = 1:14; HSV-2 (IHA) H = 1:56, C = 1:17; EBV-VCA (IFA) H = 1:131. The homosexual group also showed a higher frequency of individuals with elevated titers than the control group. The CMV IgM antibody was prevalent in 17.7% of the homosexual group and 5% of the control group; arithmetic means for ELISA values for CMV IgM were 0.207 for the homosexual group and 0.05 for the control group. In the homosexual group, the anti-CMV antibody titers increased with age (P=0.01) and with numbers of sex partners (P=0.06). Both anti-HSV-1 and anti-HSV-2 antibodies correlated with the number of sex partners (P=0.04 and P=0.05, respectively). Neither age nor partner number correlated with response to EBV, and no particular sex act was related to the EBV VCA titer level.		
14-IDB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02038-15-ID
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Combined Clinical, Viral and Immunological Studies of Peripheral and CNS Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: M. C. Dalakas, M.D. Attending Neurologist IDB, DIR, NINCDS Others: J.L. Sever, M.D., Ph.D. Chief, IDB, DIR, NINCDS M. Hallett, M.D. Clinical Director, OCD, DIR, NINCDS G. Elder, M.D. Neurologist IDB, DIR, NINCDS P. Plotz, M.D. Rheumatologist ARB, DIR, NIMSD G.H. Pezeshkpour, M.D. Neuropathologist AFIP, Wash. DC G. Di Chiro, M.D. Neuroradiologist OD, DIR, NINCDS R. Podolsky, Ph.D. Biochemist ARB, DIR, NIMSD		
COOPERATING UNITS (if any) AFIP, Washington, D.C.		
LAB/BRANCH Infectious Diseases Branch		
SECTION Immunochemistry and Clinical Investigations		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) of chronic diseases of the peripheral and central nervous system. Current studies include amyotrophic lateral sclerosis, (ALS), polymyositis/dermatomyositis, new post-polio muscle weakness, demyelinating polyneuropathies, neuromuscular complications of AIDS, certain metabolic muscle diseases and Duchenne's muscular dystrophies. Combined clinical data, and studies of virus serology and virus isolation are performed. A neuromuscular disease that occurs in patients who have had poliomyelitis at an early age (post-polio syndrome) has been clinically defined along with the rate of progression, the electrophysiological, virological and histochemical findings. The nature of oligoclonal bands found in the CSF of patients with post-polio and other chronic neurological diseases is under investigation. Patients with polymyositis are studied and the muscle changes before and after a randomized double-blind controlled study with cyclophosphamide, plasmapheresis or lymphocytophoresis are investigated. Muscle biopsies from patients with Duchenne's muscular dystrophy are studied biochemically to define abnormal proteins (i.e. nebulin). The clinical, electrophysiological and immunological picture of a chronic, sensory, "ataxic" neuropathy were defined and the presence of a ganglionopathy was found. The role of retroviruses in this neuropathy is under study in view of the finding of such neuropathy in patients with HIV and HTLV-I. The metabolic activity of the cortex in ALS patients was studied using the PET scan and ¹⁸FDG; hypometabolism was demonstrated not only in the motor but throughout the cortex, suggesting that ALS is a generalized process affecting many cortical regions. Correlation of these metabolic abnormalities with pathology of the cortical neurons is now attempted. Muscle biopsies from patients with nephropathic cystinosis and renal Fanconi syndrome were studied morphologically and biochemically. Signs of a metabolic lipid storage myopathy due to carnitine deficiency were found which prompted a therapeutic study with carnitine replacement.</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-01731-19-ID												
PERIOD COVERED October 1, 1986 through September 30, 1987 *														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Isolation, Characterization and Diagnosis of Infectious Agents from Chronic Diseases														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">P.I.</td> <td style="width: 35%;">M. Gravell, Ph.D.</td> <td style="width: 35%;">Research Microbiologist</td> <td style="width: 15%;">IDB, DIR, NINCDS</td> </tr> <tr> <td>Others:</td> <td>R.S. Hamilton</td> <td>Biologist</td> <td>IDB, DIR, NINCDS</td> </tr> <tr> <td></td> <td>M. Monzon, Ph.D.</td> <td>Virologist</td> <td>IDB, DIR, NINCDS</td> </tr> </table>			P.I.	M. Gravell, Ph.D.	Research Microbiologist	IDB, DIR, NINCDS	Others:	R.S. Hamilton	Biologist	IDB, DIR, NINCDS		M. Monzon, Ph.D.	Virologist	IDB, DIR, NINCDS
P.I.	M. Gravell, Ph.D.	Research Microbiologist	IDB, DIR, NINCDS											
Others:	R.S. Hamilton	Biologist	IDB, DIR, NINCDS											
	M. Monzon, Ph.D.	Virologist	IDB, DIR, NINCDS											
COOPERATING UNITS (if any) Section on Experimental Pathology, IDB, DIR, NINCDS														
LAB/BRANCH Infectious Diseases Branch														
SECTION Neurovirology Section														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) Human subjects</td> <td>☐ (b) Human tissues</td> <td>☒ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews					
☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither												
☐ (a1) Minors														
☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) * This project has been terminated and the remaining aspects of it transferred to project Number Z01-NS-00972-16-ID.														
16-IDB/DIR														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-01983-16-ID
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Chronic Viral Infections - Molecular Biology of Human JC Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	E.O. Major, Ph.D.	Special Expert IDB, DIR, NINCDS
Others:	K. Amemiya, Ph.D.	Special Expert IDB, DIR, NINCDS
	G.A. Elder, M.D.	Medical Staff Fellow IDB, DIR, NINCDS
	S.A. Houff, M.D.	Clinical Associate IDB, DIR, NINCDS
	W.T. London, D.V.M.	Veterinary Director IDB, DIR, NINCDS
	R. Traub	Microbiologist IDB, DIR, NINCDS
	D. Vacante, Ph.D.	Staff Fellow IDB, DIR, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Infectious Diseases Branch		
SECTION Unit on Molecular Virology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 6.0	PROFESSIONAL: 4.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our current results emphasize the molecular interactions of the JC virus (JCV) with glial cells of the human brain. Progress has been made in understanding the progression of the JCV infection which leads to a demyelination of the central nervous system of immunodeficient patients. At the molecular level we have found that JCV early gene transcription is regulated by glial cell specific proteins which recognize the DNA sequences in the JCV regulatory region. In contrast, JCV DNA replication is regulated by specific proteins which are found in a wide range of primate cells. We have provided further evidence of the accuracy of <u>in situ</u> DNA:DNA hybridization as a laboratory diagnosis for the presence of JCV DNA in brain biopsy specimens from patients with progressive multifocal leukoencephalopathy (PML). Clinical examinations, histopathology and electron microscopic identification correlated in all cases to positive hybridization results, thus indicating that hybridization tests are able to identify JCV as the cause of PML. Furthermore, viral DNA from a case study of PML was isolated and its DNA partially sequenced. The isolated virus was identified as a Mad-4 strain of JCV. From our continuing animal studies, a transplantable owl monkey astrocytoma was found in culture to release infectious JC virus. Only about 30% of the viable tumor cells in culture released virus indicating the establishment of a persistent carrier culture. The released virus designated JCV 586 was found by DNA sequencing to contain a 19 bp deletion in the second tandem repeat of the viral regulatory region, thus identifying the virus as a Mad-4 strain. In contrast to our results with the JCV Mad-1 strain, the JCV 586 T protein binds the host cellular p53 protein (a nuclear oncogene). Furthermore, monoclonal antibodies which bind epitopes at the amino terminus and the carboxy terminus of the SV 40 T protein, also recognize the JCV 586 T protein, indicating some structural homology between the JCV 586 and SV 40 T proteins.		
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02531-06-ID																					
PERIOD COVERED October 1, 1986 through September 30, 1987																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies in Neuromuscular and CNS Diseases and Their Experimental Models																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. C. Dalakas, M.D.</td> <td style="width: 33%;">Attending Neurologist</td> <td style="width: 33%;">IDB, DIR, NINCDS</td> </tr> <tr> <td>Others: J. L. Sever, M.D, Ph.D</td> <td>Chief</td> <td>IDB, DIR, NINCDS</td> </tr> <tr> <td>D. L. Madden, D.V.M, Ph.D.</td> <td>Veterinary Director</td> <td>IDB, DIR, NINCDS</td> </tr> <tr> <td>M. Gravell, Ph.D.</td> <td>Research Microbiologist</td> <td>IDB, DIR, NINCDS</td> </tr> <tr> <td>W. T. London, D.V.M.</td> <td>Veterinary Director</td> <td>IDB, DIR, NINCDS</td> </tr> <tr> <td>R. Quarles, Ph.D.</td> <td>Biochemist</td> <td>DMN, DIR, NINCDS</td> </tr> <tr> <td>G.H. Pezeshkpour, M.D.</td> <td>Neuropathologist</td> <td>AFIP, Wash. DC</td> </tr> </table>			PI: M. C. Dalakas, M.D.	Attending Neurologist	IDB, DIR, NINCDS	Others: J. L. Sever, M.D, Ph.D	Chief	IDB, DIR, NINCDS	D. L. Madden, D.V.M, Ph.D.	Veterinary Director	IDB, DIR, NINCDS	M. Gravell, Ph.D.	Research Microbiologist	IDB, DIR, NINCDS	W. T. London, D.V.M.	Veterinary Director	IDB, DIR, NINCDS	R. Quarles, Ph.D.	Biochemist	DMN, DIR, NINCDS	G.H. Pezeshkpour, M.D.	Neuropathologist	AFIP, Wash. DC
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LAB/BRANCH Infectious Diseases Branch																							
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TOTAL MAN-YEARS 1.5	PROFESSIONAL: 1.0	OTHER: .5																					
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Enzyme histochemistry in muscle and nerve biopsies</u> is carried out for diagnostic purposes in patients with several <u>neuromuscular disorders</u>. <u>Immunocytochemical studies</u> were conducted using specific antibodies to <u>thymic peptides</u> to investigate changes in the distribution of epithelial cells in the thymus of patients with <u>myasthenia gravis</u>. The interaction between cells of the <u>lymphoid and central nervous system</u> was investigated searching for common antigenic markers on their cell surface. <u>Thymosin beta₄</u>, an immunomodulating polypeptide, was found to be a common antigen shared by macrophages, dendritic lymphoid cells and oligodendrocytes. The IgM of certain patients with <u>paraproteinemic polyneuropathies</u> has been identified as a specific antibody to <u>myelin associated glycoprotein</u> or glycolipids; nerve biopsies from these patients are studied by <u>electron microscopy</u> and <u>immunocytochemically</u>. The nature of <u>amyloid protein</u> in patients with "sporadic" <u>amyloid polyneuropathy</u> was identified using specific antibodies to amyloid proteins immunocytochemically and biochemically on the extracted amyloid. <u>Immune cellular markers</u> were investigated during the evolution of EAN and EAE induced in rhesus monkeys and <u>therapies</u> were attempted using some novel <u>immunomodulating agents</u>. The mechanism of <u>inflammatory myopathy</u> in monkeys with <u>immunodeficiency (Simian AIDS)</u> due to a retrovirus D, (SRV-1), was further studied. Antibodies to SRV-1 immunoreacted with the <u>inflammatory cells invading the muscle fibers</u>; SRV-1 was capable of infecting <u>myoblasts</u> in tissue culture without exerting a cytopathic effect in the muscle. <u>Polymyositis</u> and <u>inflammatory neuropathies</u> were also identified in patients with <u>AIDS</u> and the mechanism of muscle or nerve damage is being investigated with <u>in situ hybridization</u> and immunocytochemistry. The effect of aging on the neuromuscular system of monkeys from age 5 to 25 is being investigated with a detailed morphological and morphometrical analysis of their muscle and nerve biopsies. Morphological changes in the muscles of <u>autoimmune mice</u> are also studied; <u>tubular aggregates</u> were found and their relation to <u>endogenous interferon</u> is being investigated.																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-00972-16-ID																																
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TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models for CNS Infections in Normal and Immunocompromised Hosts																																		
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A <u>simian varicella-zoster virus (VZV)</u> has been isolated from a <u>rhesus monkey (Macaca mulatta)</u> with a <u>chickenpox-like illness</u>. We refer to this new isolate as HHV. By use of <u>restriction enzyme mapping technology</u>, we have found the genomic nucleotide sequence of the HHV isolate to differ significantly from that of the previously described <u>Delta simian VZV</u>. In spite of the genomic differences found between HHV and Delta VZV, immunoprecipitation studies using specific antisera have shown homologous polypeptides from both of these isolates to share many common epitopes. Polypeptides from both of these isolates also share common epitopes with human VZV. <u>Patras (Erythrocebus patas)</u>, <u>African green (Cercopithecus aethiops)</u> and <u>rhesus monkeys</u> infected with HHV have developed a chickenpox-like illness. A severe and often fatal disease developed more frequently in patas and African green monkeys than in rhesus monkeys. The disease seen in rhesus monkeys more closely mimicked uncomplicated human chickenpox. The HHV isolate may be useful in developing an <u>animal model of human chickenpox</u>, including the <u>zoster</u> component.																																		
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ANNUAL REPORT

October 1, 1986 through September 30, 1987

Medical Neurology Branch, DIR

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1986 through September 30, 1987

Medical Neurology Branch, DIR
National Institute of Neurological and Communicative Disorders and Stroke

Acting Chief, Mark Hallett, M.D.

The Medical Neurology Branch conducts research on human epilepsy, including new approaches to diagnosis and treatment, investigates basic questions related to normal and abnormal neuronal excitability, performs studies on human motor control and speech, conducts research on Alzheimer's disease and related disorders including autonomic dysfunction, and investigates cognitive and emotional processes in man.

The Branch is divided into five Sections. William H. Theodore, M.D., is Acting Chief of the Clinical Epilepsy Section and the Neuronal Excitability Section; Mark Hallett, M.D., Chief of the Human Motor Control Section; Ronald J. Polinsky, M.D., Chief of the Clinical Neuropharmacology Section; and Paul Fedio, Jr., Ph.D., Chief of the Clinical Neuropsychology Section.

On May 11, 1987 Roger J. Porter, M.D. who was Chief of the Medical Neurology Branch, assumed the position of Deputy Director, National Institute of Neurological and Communicative Disorders and Stroke. Dr. Mark Hallett assumed the position of Acting Chief, Medical Neurology Branch. Dr. William H. Theodore assumed the positions of Acting Chief, Clinical Epilepsy Section and Acting Chief, Neuronal Excitability Section.

CLINICAL EPILEPSY SECTION

The Clinical Epilepsy Section is undertaking a series of studies using new techniques in order to improve clinical control in patients with refractory seizure problems, as well as to elucidate the pathophysiology of epilepsy. Emphasis is being placed on positron emission tomography (PET) as a technique to investigate basic mechanisms of cerebral metabolism in epilepsy and to assist in the clinical evaluation of patients with severe partial seizures. Ultrastructural and biochemical investigations of epileptic tissue removed at surgery will be correlated with metabolic findings. Magnetic resonance imaging (MRI), is being used to help elucidate the anatomical substrates of altered physiologic patterns revealed by PET.

Patients with severe uncontrolled seizures are admitted to the Clinical Center according to the following criteria: 1) patients with complex partial seizures, especially those who may be candidates for PET scan evaluation and surgical therapy or for trial of an experimental antiepileptic drug; and 2) patients with absence seizures or atonic/myoclonic for studies of cerebral metabolism including the effect of antiepileptic drugs. After seizure frequency and type is characterized by intensive monitoring techniques, the patients are placed in an appropriate research protocol. After the research protocol is completed, each

patient's therapeutic regimen is adjusted to obtain optimal seizure control. PET is a technique using the intravenous injection of a radioactive isotope to determine regional rates of cerebral metabolism. The Clinical Epilepsy Section has been using (^{18}F)-fluorodeoxyglucose (FDG) to measure the regional cerebral use of glucose. Ongoing studies include (1) patterns of cerebral metabolism in patients with partial, generalized, and atonic/myoclonic seizures; (2) the effect of antiepileptic drugs on cerebral metabolism; (3) effect of chemical seizure activation on glucose metabolism; (4) correlation of neuropsychological tests with PET results; (5) activation of normal tissue by a sustained language task; (6) use of ^{15}O water to study cerebral blood flow; and (7) use of a suitable ligand to study benzodiazepine receptors in patients with epilepsy.

The role of MRI scanning in the seizure disorders is also being investigated. MRI shows more detailed anatomic images than CT and may detect subtle changes in cerebral density resulting from small gliotic regions which may be epileptogenic.

A project has been initiated to use sphenoidal, and in some cases subdural, electrodes in the evaluation of potential surgical candidates, coupled with long-term video-EEG recording techniques. These techniques allow the acquisition of EEG data not available via surface recordings. This data is correlated with PET and MRI to obtain the best possible presurgical localization of epileptic foci. A prospective evaluation of the relative value of invasive (subdural) and noninvasive methods of presurgical evaluation is in progress.

During surgery, direct electrocorticographic recordings are made before and after tissue is resected, in order to guide the surgical approach. Tissue from both epileptogenic and nonepileptogenic regions is obtained for biochemical study.

Magnetoencephalography (MEG) is a new approach to the problem of localizing abnormal cerebral potentials which may represent an epileptic focus. Initial studies suggest that MEG may provide more precise three-dimensional information than EEG, allowing detection and localization of epileptic foci in the depths of the brain, without the need for invasive procedures.

Biochemical parameters of CSF in patients with epilepsy will be compared before and after seizures, and on changing drug regimens. Compounds to be studied include catecholamines, neuropeptides, and amino acids.

Pharmacologic studies in epilepsy continue to concentrate on studies of drug interactions and of new antiepileptic drugs. Patients with uncontrolled seizures, especially complex partial seizures or absence seizures, are accepted for study. Such patients usually have a detailed seizure calendar available prior to entering the study; they enter a week-long period of baseline determination of seizure frequency and blood levels of antiepileptic drugs while in the hospital. Each pharmacologic protocol varies, but all require modification of the antiepileptic regimen and addition of the medication under study. This may be done in single dose or chronic administration studies depending upon the particular protocol in

question. Plasma levels are often drawn daily, and on occasion, much more frequently for specific studies. Following the completion of the pharmacologic protocol, the patient is placed on a regimen which is best suited for the seizure type which has been identified by videotape/telemetered EEG analysis. This regimen is stabilized prior to discharge of the patient.

The Clinical Epilepsy Section has begun studies on a promising antiepileptic drug developed by Wallace Laboratories. The drug known as felbamate is 2-phenyl-1,3-propanediol dicarbamate. In pre-clinical testing the drug is effective in animal models which correlate with partial seizures in man and is quite nontoxic in animals. Evaluation of the protective indices indicate that felbamate has a significant and adequate margin of safety. A randomized placebo-controlled double-blind study is in progress. The purpose of this study is to obtain definitive information regarding the efficacy and safety of felbamate in patients with uncontrolled partial seizures who are receiving concomitant carbamazepine therapy. The study uses a unique three period crossover design in order to eliminate carry-over effects from drug to placebo periods.

In a separate study of patients with partial seizures, a pilot effort will be undertaken to evaluate a very old compound, dapsone, for its effect on control of partial seizures. This drug has been used for decades in the treatment of leprosy as well as in dermatologic disorders; more recently it has demonstrated efficacy in animal models--such as the maximal electroshock--and its protective index is promising. This pilot study will not be a controlled clinical trial but will evaluate the pharmacology of the drug in humans, the drug interactions with current antiepileptic drugs, and will obtain some preliminary assessment of efficacy of the drug.

Still underway is a study evaluating the relative potencies of a widely used antiepileptic drug, carbamazepine, and its metabolites, especially carbamazepine 10-11 epoxide. This study, which evaluates both toxicity and seizure control, may prove valuable in designing optimal antiepileptic drug treatment regimens because the role of the metabolite will be better understood.

Several new studies of autonomic function in patients with partial seizures have been initiated. Cardiac arrhythmias and neurogenic pulmonary edema may be significant risks in this patient group.

HUMAN MOTOR CONTROL SECTION

The mission of the Section is to understand principles of normal motor control in man and the pathophysiology of motor disorders including both deranged voluntary movement and involuntary movement. The Section is composed of a Speech Pathology Unit and a Movement Disorders Unit.

Speech Pathology Unit

The goal of the Speech Pathology Unit is to determine the neurological organization of speech and phonatory control through the study of the

breakdown of these functions in neurological disorders. The research continues to address three major issues: (1) The neurophysiological bases of phonatory disorders; (2) Speech production timing as evidenced in neuropathologies of speech; and (3) The brain organization of speech production and speech perception from brain lesion studies.

Phonatory Function: To determine the pathophysiology of spasmodic dysphonia, neurophysiological techniques were developed for recording with concentric needles from two intrinsic laryngeal muscles, the thyroarytenoid and cricothyroid. A new laboratory computer system was developed and the signal processing programs completed. The first study completed this year compared laryngeal muscle activation patterns during quiet respiration and phonation in spasmodic dysphonia and normal controls. Maximum levels of muscle recruitment prior to, at onset and during phonation were higher in the patients. However, the percent increase in activation over resting levels were the same in the patients as the controls for the thyroarytenoid muscles. Therefore, increased muscle tone was present both at rest and during phonation in the patients. Further, the activation ratios of the cricothyroid muscles were higher than in the thyroarytenoid muscles in the controls but not in the patients. Thus, muscle recruitment levels and patterns of activation between the laryngeal muscles differed between the two groups.

To determine whether spasmodic dysphonia is a task specific focal dystonia, muscle activation patterns of the two groups were also compared during quiet respiration. Both minimum and maximum activation levels during respiration were significantly greater in the patients. Therefore, laryngeal muscle tone and activation levels were increased in spasmodic dysphonia over normal in other tasks besides phonation. A temporary unilateral recurrent laryngeal nerve block was used to determine whether muscle activation patterns on the opposite side of the larynx are reduced with a reduction in muscle spindle feedback. In the first four patients, activation was reduced during respiration on the opposite side following the nerve block. However, during phonation activation increased on the side opposite the block possibly due to the need to overadduct the unaffected side to achieve vocal fold closure for phonation. Additional patients have been tested and their data is being analyzed.

Testing is ongoing on two additional studies. In one, to determine the effects of a central depressant on muscle activation levels in spasmodic dysphonia, the effects of diazepam on muscle activation patterns in patients and controls is being determined both at rest and during phonation. Thus far, the data suggest a depressive effect on laryngeal muscle activation in normals and not in patients. In another, to determine whether recurrent laryngeal nerve surgery alters the pathophysiology of spasmodic dysphonia, the activation levels on the unoperated side of patients who have had unilateral recurrent nerve sections are being compared with those of spasmodic dysphonic patients who have not had surgery. Reinnervation has been found in all four post surgical patients studied thus far, with reduced activation levels on the unoperated side. These studies suggest reduced central inhibition of

laryngeal muscle activation in spasmodic dysphonia with increased sensitivity to muscle spindle feedback. Studies examining the effects of electrical and auditory stimulation on muscle activation are planned to further examine the pathophysiology of this disorder.

Since botulinum toxin injections have been successful in treatment of other focal dystonias such as blepharospasm, two trials of botulinum toxin injections for the temporary alleviation of phonatory spasms were conducted this year. In the first trial, single 10 Units/.1 ml injections into the left thyroarytenoid were administered and followed two weeks later by 10 Units into both the left thyroarytenoid and cricothyroid muscles. This was repeated again 2 weeks later if no effect on vocal fold movement was seen. Three patients participated; two who previously had surgery of the left recurrent laryngeal nerve were injected on that side and one with no previous surgery. One patient, the mildest, had a response after the second injection. Vocal fold movement on the injected side was reduced within 2 days following injection and the voice remained aphonic for 5 days. With gradual voice return, few breaks occurred in phonation for 35 days with symptoms returning within 45 days.

In the second trial, 10 Units of toxin were injected at reduced concentration (5 Units/.1 ml.) in 6 different sites within the left thyroarytenoid muscle on the first injection and 15 Units on second injection, 2 weeks later. Five of the 6 patients had reduced vocal fold movement and a change in voice 2 days following the second injection. Only the most severe patient did not have a response with no change in vocal fold movement on the injected side. In the patients with a beneficial response, the voice first became breathy but returned within 7 days with reduced phonatory breaks. This improvement remained for 4-5 weeks with symptoms gradually returning in the 6th week. The patient's report continued ease of phonation into the 7th week. The only negative side effect has been some swelling in the laryngeal area interfering with movement speed during swallowing of liquids. This starts within 3 days after the second injection and can continue up to 7 days. Laryngeal electromyography is being conducted pre- and post-treatment in these patients to determine whether this treatment alters the pathophysiology seen in spasmodic dysphonia. Objective methods of quantifying phonatory function in spasmodic dysphonia over time were developed and will be used to evaluate patients pre- and post- botulinum treatment.

Speech Timing: An acoustic study of speech in Parkinson's disease patients speech completed this year found no difficulties with place of articulation contrasts in a controlled experimental setting, although their speech was perceptually impaired when not experimentally controlled. A new series of studies were initiated this year as an outgrowth of this research and of our previous acoustic studies. The purpose is to examine the effects of linguistic frequency, length, and preplanning on speech production in both normal adults and patients with Parkinson's disease. The timing of speech initiation, movement velocity, muscle activation patterns and articulator coordination for respiratory, lip, jaw and laryngeal movement are being measured to determine the effects of different linguistic encoding demands

on speech production. It is hypothesized that patients with dysarthria associated with neurological disease are most affected in the enactment of speech movements in complex and unfamiliar utterances requiring online planning and organization. This might explain their poor speech intelligibility in conversational settings in contrast with controlled testing situations when their speech skills are close to normal.

Neurological Organization of Speech and Language: Analyses of speech and language data from the Vietnam Head Injury Study are continuing to address the neurological organization and inter-relationships between speech production, speech perception and language. These projects are aimed at determining the lesion topography of penetrating missile wounds underlying specific chronic deficit patterns 15 years post injury. Two subtypes of persistent speech dyspraxia were studied; one with associated nonfluent aphasia, the other without any language deficits. When contrasted with other penetrating brain lesion locations in patients without speech/language deficits, all speech dyspraxia patients had left cortical involvement of the primary motor strip (area 4), partial extension to areas 6, 8 and 9 and deep extension to the insula, superior longitudinal fasciculus, frontal corona radiata and the anterior corpus callosum. Experimental speech testing demonstrated that despite large lesions involving the pyramidal tract and cortical regions associated with speech production in the left hemisphere, some patients had only mild chronic residual speech deficits suggesting considerable reorganization for speech motor control following penetrating missile wounds in young men.

Movement Disorders Unit

The Movement Disorders Unit has now been active for about two years. A number of projects are in progress.

Studies using the Gait Laboratory of the Department of Rehabilitation Medicine have focused on the control of balance. Simultaneous measurement of body angles, foot-floor forces and multiple EMG's are possible. Studies in normal subjects have revealed insights into the biomechanical effects of postural muscle activity. Similar studies have been initiated in patients with cerebellar disturbance and Parkinson's Disease. Two patients with orthostatic tremor have also been investigated.

In patients with Parkinson's Disease there was a positive correlation between circulating levels of dopamine and cognitive motor capabilities in a choice reaction time situation but not in a simple reaction time situation. In normal subjects, triggered muscle responses produced by stopping voluntary movements were similar to muscle responses generated when subjects initiated a voluntary movement from the stopped location, thereby suggesting that triggered and voluntary reactions are mediated by similar central nervous system mechanisms.

The study of motor control in hemiplegia is being planned, and apparatus is being prepared. Patients with discrete brain lesions will be studied; patients with strokes will be the main group, and many will be followed

serially from onset of the disorder to recovery. Preliminary studies with positron emission tomography (PET) of normal subjects have shown that it is difficult to identify cortical areas active with voluntary movement using (^{18}F)-fluorodeoxyglucose (FDG) as the tracer. Further studies are planned with oxygen-15 water which will probe blood flow rather than glucose metabolism.

Myoclonus and a number of other rapid involuntary movements have been difficult to classify clinically. Clinical and physiological analysis of a continuing series of patients has led to new classifications and pathophysiological insights. A series of patients with epilepsy partialis continua have been studied; the pathophysiology is heterogeneous, but, in most, electrophysiological techniques can identify an excitable region in cerebral cortex. A series of patients with adult onset tic have been analyzed and clinical rules have been formulated to make this diagnosis. Positron emission tomography (PET) studies of patients with palatal myoclonus have revealed that the inferior olives are hyperactive. PET studies of patients with hemiballismus have revealed that hypoactivity of the ipsilateral striatum occurs as a result of the lesion in the subthalamic nucleus.

In studies of postural action tremors, we have been analyzing the amplitude of the tremor as a function of the precise posture of the limbs. For many tremors, such as cerebellar postural tremor, the tremor is worse when the arms are near the body, and the hands are pointing towards each other. For other tremors, this is not true, and the clinical and physiological significance of this is being explored.

Task specific focal dystonias of the hands, such as writer's cramp and pianist's cramp, have been analyzed, and a number of physiological characteristics have been defined. There appears to be diminished ability to control the fingers independently, and gating of somatosensory evoked potentials with voluntary movement is abnormal. The spasms themselves have been characterized into different patterns. Abnormalities of the blink reflex have been identified in dystonic disorders. We have verified this in a number of our own patients, and are now applying this test to the patients with focal hand cramps.

Recently techniques have become available for the non-invasive stimulation of the human cortex and human spinal cord. A device has been manufactured which produces a high voltage, extremely brief pulse which can penetrate skull and activate central nervous system without excessive pain. Previous studies have shown it to be useful in some circumstances where evaluation of the speed and integrity of motor pathways is valuable.

We have just begun to use this technique. Our first project is to see if we can map the motor cortex.

Previous studies have shown utility of isoniazid for ameliorating cerebellar postural tremor in patients with multiple sclerosis. Current studies are aimed at identifying whether patients with action tremors of

other types are also benefitted. A double-blind placebo-controlled, cross-over trial is in progress. Thirteen patients have now been studied. Isoniazid does appear to help some of these cases, but we do not have enough experience yet to predict the response.

Botulinum toxin injected in small doses directly into muscle binds to the neuromuscular junction, and inactivates it for approximately 3 months. This treatment has been demonstrated to be useful for strabismus and blepharospasm, but there has not been a complete understanding of its mechanism of action.

Studies of utility of botulinum toxin have been carried out in spasmodic dysphonia and writer's cramp (and its variants, such as pianist's cramp). Treatment appears efficacious for both. Further studies are planned with adult stuttering.

Studies of physiology of the mode of action have been carried out in spasmodic dysphonia, writer's cramp, blepharospasm and hemifacial spasm. These studies are currently incomplete.

CLINICAL NEUROPSYCHOLOGY SECTION

Cognitive and emotional activities in man are dependent in part, upon the integrity of the fronto-limbic system, and therefore, insult to these brain regions affords neuroscientists a unique opportunity to examine and to better understand the neural basis of human behavior. In a series of studies, memory, perceptual and emotional processes were evaluated by behavioral and electrophysiological techniques in patients with neurological and neuropsychiatric disorders involving cortical and subcortical regions.

Several studies by members of the Section continue to strengthen impressions of an asymmetry in the organization and expression of mood and emotions by the left and right limbic systems. For example, scenic materials which were ranked as pleasant or horrific by normal subjects were rated as less affective or as neutral by left temporal lobectomy patients, and with inappropriate verbal descriptions. The same response bias held for the left temporal patients in judging photographs of faces displaying different emotional expressions. Overall, there were fewer judgmental errors by patients following right temporal resection. Moreover, the left and right temporal patients were physiologically unresponsive while viewing affective material, as indexed by skin conductance indices. Unlike normal subjects, the temporal lobectomy patients were unable to take advantage of the "emotional" information and to improve subsequent memory and recall.

In an independent neuropsychological study, veterans with penetrating brain

wounds involving orbito-vs-dorsofrontal or nonfrontal cortex were examined by observed and self-reported indices of mood and cognition. Overall, patients with right and bilateral orbitofrontal lesions reported the highest rate of maladaptive behaviors and need for psychiatric treatment. Many of these patients reported increased anger/combative behaviors and the emergence of traits as "edgy ... reactive". In contrast, patients with left orbitofrontal lesions acknowledged fewer psychological difficulties. Parenthetically, persistent anger and hostility were more readily assigned to patients with left dorsofrontal lesions. The study also emphasized the role of intelligence, family, environment and pretraumatic predisposition in shaping post-traumatic behavioral residua.

In an effort to assess the value of compensatory strategies for amnesiac defects following temporal lobectomy, patients were instructed in the use of different encoding cues. The study confirmed the facilitatory effects of visual imagery. In a related study of enhancement by motor encoding, subjects received instructions to either print a word or to sketch a picture of an object represented by a word, overtly (graphically) or covertly ("in mind's eye"); whereas all groups remembered fewer printed than sketched words, the left temporal group did less well to remember the sketched material.

In another mnemonic paradigm utilizing associative phonetic, spatial, or praxic cues, all groups, particularly the left, did very poorly with phonetic encoding. In contrast, spatial and praxic mnemotechnics proved beneficial, more so for the left temporal patients. These data confirm that left temporal mechanisms are indispensable to encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategies that combine overt or covert imagery with praxic encoding. Parenthetically, this paradigm will be used with dementia patients to assess the efficacy of motoric encoding as a means of reducing memory impairment.

Neurobehavioral changes have also been examined with computer derived, electrophysiological indices. Procedures were developed to analyze neural components of cognitive or judgmental processes (P300 events). During the past year, considerable effort has been devoted to identifying critical parameters which influence the P300. As a result, a model has been formulated, suggesting that P300 amplitude is a function of two factors, viz., subjective probability and stimulus meaning which are modulated by the amount of stimulus information that is perceived by the subject. In addition, the P300 latency was found to index the time required by the subject to extract information about the stimulus. These exciting results promise to greatly increase the diagnostic utility of the event-related potential (ERP).

While neural generators for ERP components have been advanced in animal studies, efforts to explore the hypothesis are limited in human subjects. Based on preliminary data from indwelling, diagnostic electrodes in epileptic patients, investigators have argued that the P300 component may

be generated by temporal lobe structures. In this context, and in collaboration with neurologic surgeons, temporal lobectomy patients were available, and provided a unique opportunity to examine this model. To date and based on a small number of patients, we have found no support for this hypothesis or for that matter, consistent hemispheric asymmetries that distinguish left and right temporal patients or either group from control subjects. An intriguing result from the lobectomy patients, however, was the subtle differences in ERPs, dependent on the modality of the eliciting stimulus. That is, left temporal patients showed normal activity in response to visual events while their ERPs to auditory stimuli showed some abnormalities; this pattern was reversed in patients after right temporal lobectomy.

In a study with a developmental neuropsychological bent, maturational changes reflected in various components of ERP were examined in normal children, 7 to 20 years of age. Most striking was the altered nature of frontal negative slow waves. In seven year-old children, for example, this wave was much larger (approximately 20 uV) and longer in duration (i.e., from 80-900 msec post-stimulus). P300 activity was also affected by age, with latencies decreasing by more than 100 msec during the continuum of child-adulthood (ages 7-20 years).

ERP activity in young normals and patients with metabolic disorders such as Turner's syndrome was similar. In contrast, differences became more evident during and after puberty, particularly for the frontal negative slow wave activity which was elicited 80-900 msec after stimulus onset. In fact, the waveforms from some of the 18 and 20 year-old Turner patients resembled those from normal younger children (at the age of puberty). These results lend credence to impressions that hormones assume a major role in brain development and in the evolution and support of distinct cognitive profiles.

Interest has also focused on dementia, and in one electrographic study, patients with Alzheimer's disease (AD) were assigned to different subgroups, depending on a pattern of verbal vs. visuospatial deficits. Procedures were developed to monitor the deteriorative course of linguistic, spatial, and short-term memory functions in AD patients, as well as patients with Progressive Supranuclear Palsy (PSP), a form of sub-cortical dementia. The tests monitored processes which occur automatically in normal persons, thereby allowing these functions to be followed as the patients become progressively worse. A second goal of this experiment involved comparing the pattern of ERP changes in sub-cortically demented patients to localize the neural generators of the ERP components, and the effects of dementia on physiological activity. Pilot work has revealed the optimum parameters for each paradigm and data collection has begun.

In the neurobehavioral domain, visual, spatial, and constructional abilities were examined with neuropsychiatric patients with Alzheimer's or

Huntington's (HD) disorder. The pattern of deficits was different; HD patients exhibited relatively greater impairment on tests of spatial judgment. These findings, viewed in the context of studies of patients with frontal vs. parietal lobe lesions, implicate frontal striatal mechanisms in HD, and cortical regional changes in AD. The central theme of investigations with neuropsychiatric patients indicated that, at least during the early stages, AD patients may present with qualitatively different cognitive profiles and patterns of decline corresponding to different regions of neuropathology. Thus, questions concerning the status of cognition and memory in these patients cannot be meaningfully or adequately addressed if AD is treated as a homogeneous disease entity.

There is convincing evidence that many AD patients enjoy varied and relatively impaired vs. spared intellectual functions. For example, in a recent study of a large cadre of AD patients (N=42), relatively young (59.3 years average), well educated (14.5 years of education) and with mild symptoms of recent onset (3.3 years), several groups were statistically identified, each characterized by a distinct neuropsychological dysfunction. Specifically, one group of AD patients exhibited widespread and uniform reduction of linguistic and visuospatial defects. The remaining 2 groups had a marked asymmetry; that is, better functioning in verbal than visuospatial domains, or the converse. Correlative profiles with positron emission tomography (PET) supported the impression of bilateral asymmetrical hypometabolism in the left or right temporal-parietal regions.

We are currently undertaking a large scale, computer analysis (involving over 40 cognitive measures) to better characterize the "subgroups", and to improve the reliability. For select patients, a similar analysis is being carried out on data from positron emission scanning in a bid to relate altered neural state with observed neuropsychological deficits, and to advance a more precise understanding of brain-behavior relations in man.

In a related study, three AD patients with distant deficits, semantic and spatial knowledge were examined. One patient with a neurolinguistic disorder enjoyed relatively preserved visuospatial and constructional skills, whereas this functional asymmetry was reversed in another patient; the third patient showed deficits in both domains. There was selective loss in semantic knowledge with either impaired or intact drawing and constructional skills, depending on the stimulus model or standards to be reproduced. Analysis of semantic and spatial knowledge in these patients suggested that cognitive learning and memory depend upon the integrity of different neocortical systems to service skills or knowledge. These systems support on-line processing of specific materials and recall/reproduction in the absence of the recalled stimulus. Specifically, temporal lobe structures provide an associative and integrative role in ensuring access and recall of engrams from memory stores.

Extending these studies pharmacologically, behavioral changes by

Parkinsonian patients revealed subtle but consistent cognitive changes with "on-off" phenomenon, and residua after long-term treatment with anti-Parkinsonian drugs. On the one hand, the profound motor effects of this disorder are thought to be reflective of functional depletions in the nigrostriatal/dopaminergic pathways. The observed cognitive effects, on the other hand, tend to implicate hypofunctionality of mesolimbic/cortical dopaminergic pathways. Thus, dopamine neurotransmitters are important to delayed memory function, particularly in the realm of effortful memory. This assertion has been supported by animal data as well as clinical observations, and offers further credence to the hypothesis that specific neurotransmitter systems may function as necessary but not sufficient modulators for cognitive function.

The hypothesis that successful restoration in deficient neurotransmitter systems in neuropsychiatric disorders provides symptomatic relief was studied by inducing pharmacologic restoration of several neurotransmitter systems reportedly deficient in AD. As an example, the efficacy of THIP [4,5,6,7-tetrahydroisoxazolo(5,4,-c)pyridin-3-ol], a potent and specific GABA agonist, was assessed with neuropsychological procedures; no significant improvement was ascertained. Cholinergic neurotransmission in AD was also examined with an investigation of the muscarinic agonist RS-86. Again, although doses were increased to maximally individually tolerated levels, no consistent clinical improvements could be discerned.

Recent work with experimental animals suggested significant noradrenergic involvement in deficits associated with normal aging; the noradrenergic system is also reportedly deficient in AD. To examine the utility of noradrenergic therapy in AD, we are currently undertaking a study with the potent alpha-2 receptor agonist clonidine. Preliminary analysis of 4 patients with relatively severe dementia did not produce encouraging results. However, a trial is presently underway with AD patients with mild dementia to examine the possibility that insufficient neuron populations in the more severely affected patients did not allow for some restorative action of this medication. To encourage a more targeted approach with pharmacologic restoration of deficient neurotransmitter cholinergic systems, we have also challenged neuronal system with scopolamine, to study the role of acetylcholine in cognition and memory, and to allow for the development of prognostic tests of efficacy of specific therapeutic approaches, such as THA, a metabolic degradation blocker of acteylcholine; the study remains incomplete.

Behavioral and neuropharmacologic studies of Parkinson's disease were undertaken as well. Preliminary data suggest that dopaminergic neurotransmission plays a role in the manifestation of this disease above and beyond the familiar motoric concomitants. Dopaminomimetic therapy can evidently attenuate, but not restore circumscribed cognitive deficits exhibited by the untreated patient, most notably in the area of delayed and effortful memory. All observed effects were minimal, indicating that this therapeutic approach alone is insufficient to alleviate cognitive deficits in PD.

The beneficial effects of L-Dopa therapy on some aspects of memory is complicated by "paradoxical" responses of individual patients to dopaminomimetics, namely, some patients evince cognitive improvement in an untreated state. This seeming inconsistency raised questions about overmedication of cortical sites, where DA levels were notably less deficient than in subcortical systems. We are presently examining questions about dose response curve with L-Dopa infusion in PD patients.

Our understanding of cognitive defects associated with Parkinsonism are hindered by variability in the severity and nature of neuropsychological deficits. To address this issue, we adopted a three tier approach to assess deficits in different PD groups: (1) patients suffering from left or right hemiparkinsonism are being compared to determine whether cognitive deficits show the same kind of functional-cortical lateralization pattern as the motor signs, or whether cognitive changes remain separate and distinct; (2) high, intelligent functioning PD patients in leading positions in government, academia and industry are studied to identify the source of a common underlying complaint by patients about slowed mentation and an inability to arrive rapidly at critical decisions. To examine possible frontal lobe deficits, we are currently examining executive, visuospatial, verbal, mnemonic and attentional functions in this group of patients; no conclusive data are available; (3) in certain Parkinsonian individuals, cognitive deficits extend well beyond the variable, but generally circumscribed, cognitive deficiencies. To examine the nature of this dementia, we are comparing neurobehavioral profiles of PD or Alzheimer's dementia. Early data analysis supports distinctions, but at this point, it remains unclear whether differences outweigh commonalities.

In a newly formulated study, individuals at risk for Alzheimer's disease are being studied by neuropsychological procedures to identify early functional markers for changes in brain-behavior relations. This study examines semantic and episodic memory processes, but has not yielded data for analysis.

The emergence of depression in normal elderly frequently mimics dementia, posing a diagnostic enigma and challenging treatment. A series of studies relevant to this issue are in preparation, intending to use psychophysiological and electrographic techniques to differentiate affective and neuropsychiatric patients and disorders.

Cerebral dysfunctioning of frontal mechanisms has also been implicated in obsessive-compulsive disorders with genetic linkage to Huntington's disorders. Guided by this hypothesis, a series of perceptual and memory tasks were developed and administered to preadolescent and adolescent obsessive-compulsive patients (N=26) and matched normal subjects (N=24). The patients consistently did poorly with spatial learning and memory tasks, and procedures requiring left-right directional judgments or imaginary self-rotation in space. Organizational defects and atypical strategies were commonly observed with the patients. The obsessive-compulsive patients also showed elevated thresholds for visual material

tachistoscopically projected to the left, right, and central fields, and a sharp ear asymmetry with dichotic recall. These data were interpreted in the context of regulatory inhibition-dysinhibition. Thus, defective frontal neural regulators may propel automatic-stereotypic expressions of ideative and ritualistic behaviors in obsessive-compulsive disorders. To date, about half of the obsessive-compulsive subjects and controls have been reevaluated. Preliminary results with select tasks showed that although both groups performed significantly better on retest, the relative discrepancy between the normal and obsessive-compulsive group remained essentially unchanged.

The alleged genetic linkage between obsessive-compulsive disorders and other neuropsychiatric disorders also serves as a background for Tourette's Syndrome. Patients with this atypical, neuropsychiatric disorder showed impairment on some of the same tasks that troubled the obsessive-compulsive patients. Since similar deficits have been observed in adults with frontal lobe dysfunction, it was speculated that an imbalance in the inhibitory mechanisms of frontal or frontally connected systems contribute to symptoms common to obsessive-compulsive, Huntington and Tourette's disorders. Eventually, a multifactorial discriminant analysis may yield a more precise picture.

NEURONAL EXCITABILITY SECTION

The Neuronal Excitability Section is undertaking a series of studies to determine whether the breakdown of the cytoskeletal protein fodrin is involved in the seizure focus. Emphasis will be placed on studying the structure of fodrin through antibody and electrophoresis techniques.

The question of the involvement of fodrin is addressed at several levels comparing epileptic focus to normal brain in each case:

1. Measurement of fodrin, for which gel electrophoresis and antibody techniques are used.
2. Calcium influx for which synaptoneurosomes preparations in conjunction with radioactive calcium will be used.
3. Calpain the protease which breaks down fodrin will be evaluated by the ^{14}C -casein breakdown technique.
4. Measurement of resting membrane potentials will be carried out in synaptoneurosomes using the ^3H -tetraphenyl phosphonium technique.
5. Measurement of glutamate receptors on the synaptoneurosomes will be performed by the binding of ^3H -4-amino phosphorobutyric acid.

Synaptoneurosomes were prepared from kindled and sham operated rats. The brain parts used were amygdala, substantia nigra, cortex, olfactory bulb and hippocampus. Using the ^3H TPP⁺ methodology membrane potentials were

found to be in the expected range of -60 to -70mV. However under the experimental conditions used, we were unable to demonstrate any differences in membrane potentials in the kindled vs sham operated rats. The failure to demonstrate a difference may be due to several factors: 1) exposing the tissue to non physiological conditions during the preparation of the synaptosomes may have destroyed any inherent membrane potential differences; 2) the membrane potential difference is localized to a small subgroup of neurons and hence cannot be detected in a more crude preparation of whole amygdala, cortex, etc. Therefore, the experiments are being tried in several other ways which may yield increased sensitivity and better detection: a) infusion of the rat brain with $^3\text{(H)TPP+}$ following the breakdown of the blood brain barrier with mannitol, followed by quantitative autoradiography, b) infusion of the brain with voltage sensitive dyes followed by visualization under a fluorescence microscope. While the TPP+ assay for membrane potential was being set up this compound was tested in the presence of a number of neurotransmitters to determine whether the changes in uptake of TPP+ reflected the known electrophysiology of these compounds. Depolarization was detected in the presence of arecoline, glutamate and VIP. Hyperpolarization was detected in the presence of muscimol. The one exception among the compounds tested was Substance P. This compound caused hyperpolarization of the synaptoneurosome membrane as opposed to the expected depolarization. When the rats were treated reserpine, and synaptoneurosome prepared the excitatory effect of Substance P could be detected. This finding indicates that a catecholaminergic could be involved in the hyperpolarization mediated by Substance P. It is also an indication that the system may be used for the study of the mechanisms of transmitter action. Several antiepileptic drugs were also tested for their effects on the membrane potential in synaptoneurosome. At concentrations of 10^{-10} to 10^{-6}M phenytoin hyperpolarized the membranes, whereas at 10^{-6} to 10^{-5}M phenytoin depolarized the membranes. This observation is similar to that reported in the literature following *in vivo* studies. Carbamazepine from 10^{-10} to 10^{-5}M had no effect on the membrane potential. Valproic acid gave a 20mV hyperpolarization at 10^{-7}M . The TPP+ method appears to be an interesting way of investigating neurotransmitter and drug effects and further studies along these lines are underway.

A gel electrophoresis method using 7.5% polyacrylamide gel was established to determine fodrin. A doublet which stains with coomassie blue at the 240 000 MW appears at the synaptic membranes prepared with SDS. This spot corresponds to the location of fodrin published in the literature. The identity of this protein will be double checked by immunoblotting. Once this is done rat brain dissected during level 5 seizure will be checked for differences in fodrin in the intact animal versus the seizing animal. In addition, similar studies are being undertaken with fodrin conjugated to rhodamine dye where this compound then can be observed under fluorescence microscopy.

Amino acids, catecholamines and neuropeptides were determined in the focal and nonfocal areas from the temporal lobe of patients with intractable epilepsy. The amino acids determined with the Beckman automated amino acid

analyzer showed no differences between the focus and nonfocus. However, differences could still exist in the turnover of these amino acids from slices.

Catecholamines showed some interesting differences. Norepinephrine, dopamine and dopa were all increased in the epileptic focus versus the nonfocus. The following values were obtained from 25 patients (mean $\pm$ SD): DOPA 24.8 ± 5.3 ng/g in the focus vs 9.5 ± 4.0 ng/g in the nonfocus ($p < 0.01$); dopamine 14.6 ± 11.4 ng/g in the focus vs 6.1 ± 3.7 ng/g in the nonfocus ($p < 0.01$); norepinephrine 19.7 ± 11.3 ng/g in the focus vs 9.3 ± 6.2 ng/g in the nonfocus ($0.05 < p < 0.1$). These findings agree with the findings of Sherwin et al., who showed that tyrosine hydroxylase was elevated in the epileptic focus. This is the first time that these catecholamines have been measured in the human cortex.

The glutamate and aspartate levels, which are elevated in the spiking tissue may indicate a more excitable focus. The elevated levels of glycine may show that this amino acid may be interesting with the NMDA receptor and making the focus an electrophysiologically irritable area. A number of peptides were also measured in the spiking and non spiking tissue from 25 epileptic patients. The most interesting observations to emerge from these measurements are the elevation of neuropeptide Y and somatostatin. The elevation of somatostatin has been noted by us and by others in the kindled rat model as well. The correlations between somatostatin and seizures is yet unclear. Studies are underway in our laboratory to determine dynamic interactions between somatostatin, catecholamines, and amino acids with in vitro perfusion techniques.

Spiking tissue from the human epileptic brain has also been investigated for enzymes involved in neurotransmitter synthesis. In connection with the lack of alterations in GABA levels in the focus we also failed to see differences in glutamate decarboxylase. As mentioned earlier tyrosine hydroxylase was elevated in the spiking tissue as was choline acetyltransferase. The human cortex was also evaluated for Na^+ , K^+ ATPase. No differences in the levels of this enzyme were observed.

Several receptors were also evaluated in the human epileptic focus. Among these were GABA, benzodiazepine, acetylcholine, NMDA, alpha, and beta receptors. A decrease in alpha and beta receptors were seen. The decrease in the number of cholinergic receptors may be a result of the increased number of acetylcholine molecules.

A new focus of research within the Neuronal Excitability Section is directed toward using neurophysiological techniques to:

1. Understand basic mechanisms of excitability within the mammalian CNS by a detailed study of membrane ionic conductance systems,
2. Determine the precise mode of action of clinically relevant drugs that interact with ion channel mechanisms, and

3. Study the activity of neurons during states of abnormal electrical excitability, such as epileptic seizures.

During the reporting period facilities to carry out these studies were established. These consist of a system for patch clamp recording from cells in monolayer culture or acutely dissociated from brain slices and equipment for single cell recording in the restrained rat during experimentally-induced epileptic seizures.

Experimental work using the patch clamp facility was directed toward characterizing voltage-dependent conductances in several clonal cell lines. Studies were carried out on potassium conductances and on a novel calcium-dependent chloride conductance. Information was obtained at the level of macroscopic currents using the whole cell voltage clamp technique and by recording single channel activity using the patch technique. These conductances serve as models for studying the effects of clinically relevant drugs on defined ionic channel systems. A project was completed demonstrating the specific effects of aminopyridines, a novel class of convulsant drugs, on the potassium conductances in clonal pituitary cells. Work was also completed on the effects of tetrahydroaminoacridine, a drug recently shown to have beneficial effects in Alzheimer's disease, on voltage-dependent conductances in cultured hippocampal neurons.

A technique was developed for the isolation of neurons from adult mammalian brain by enzymatic dissociation of brain slices. These cells were found to be suitable for patch clamp recording. A detailed study of the ionic currents and responsiveness to neurotransmitters of hippocampal and thalamic (dorsal lateral geniculate) neurons is in progress.

Using the in vivo recording unit, studies are in progress characterizing the response of CNS noradrenergic neurons in the locus coeruleus (LC) during experimentally-induced epileptic seizures. Preliminary results have revealed that LC neurons show distinctive changes in their firing activity in response to seizures. These results help to explain the biochemical changes that have been observed in central norepinephrine (NE) systems in animal seizure models and in epileptic foci from human patients; they provide a basis for understanding the anticonvulsant role of brain NE neurons; and may provide insight into the changes in receptor sensitivity that occur with spontaneous or induced seizures (electroshock).

CLINICAL NEUROPHARMACOLOGY SECTION

The Clinical Neuropharmacology Section continues to develop clinical, physiological, biochemical and pharmacological methods for assessment of autonomic nervous system function in man. Autonomic failure may occur alone (pure autonomic failure, PAF) or in association with central nervous system degeneration (multiple system atrophy, MSA). Investigation of patients with lesion(s) of the autonomic nervous system provides an opportunity to examine the interaction between the autonomic nervous system

and other hormonal/peptide systems.

Since norepinephrine is the neurotransmitter released by most post-ganglionic sympathetic neurons and is also an important central nervous system neurotransmitter, our investigations have focused primarily on the noradrenergic system. High performance liquid chromatography, liquid scintillation spectrometry, and gas chromatography-mass spectroscopy are used to measure neurotransmitter and metabolite levels in plasma, urine, and cerebrospinal fluid. Peptide and hormonal levels are determined by radioimmunoassay. These substances are measured under basal conditions and after a variety of stimuli have been applied to elicit a sympathetic nervous system response.

We have continued to investigate insulin-induced hypotension since endogenous insulin release may play an important role in causing post-prandial hypotension which may have serious practical and medical consequences. Our previous work showed that insulin-induced hypotension was not due to excessive beta-adrenergic stimulation. However, the mechanisms involved in producing this effect of insulin may differ between PAF and MSA. The consequences of hypoglycemia have been separated from other insulin effects by using a glucose-clamp technique. Infusion of glucose partially prevents insulin-induced hypotension in MSA but not PAF. In addition, there is a correlation only in MSA patients between the ratio of norepinephrine and beta-endorphin responses during hypoglycemia and the hypotension observed following insulin administration. Insulin-induced hypotension in MSA may result from release of endogenous beta-endorphin which only occurs if the hypoglycemia is not prevented. This hypothesis was further tested by repeating the insulin test during infusion of naloxone, a narcotic antagonist capable of blocking the hypotensive effect of beta-endorphin. Although naloxone pretreatment caused a significant drop in mean blood pressure in MSA, it also reduced the further blood pressure decrement during insulin hypoglycemia. Post-prandial hypotension may result from endogenous insulin release in response to a meal since patients with autonomic failure manifest a significant reduction in blood pressure during a euglycemic insulin test. Further studies of the hypotensive effect of insulin will include measurements of peripheral vascular resistance and venous compliance.

Deficient cortisol responses to insulin-induced hypoglycemia and normal ACTH responses to metyrapone in MSA patients suggest the possibility of abnormal glucoreceptor function. However, we have shown that the beta-endorphin response to hypoglycemia is reduced in MSA. Since beta-endorphin and ACTH are derived from the same precursor, it may be that these responses are dissociated under certain circumstances. Alternatively, there may be a poor correlation between ACTH and cortisol responses to the same stimulus. We are presently comparing the ACTH and beta-endorphin responses during insulin hypoglycemia with those observed following infusion of arecoline, a centrally active muscarinic agonist which stimulates release of beta-endorphin and cortisol. Preliminary results suggest that beta-endorphin responses to arecoline are deficient in MSA patients, consistent with a defect in synthesis and/or release rather than

an abnormality of glucoreceptor function. Hypothalamic degeneration in MSA is attended by a reduction in choline acetyltransferase, a marker of cholinergic neuronal integrity. Destruction of this cholinergic pathway may be the basis for the decreased beta-endorphin responses in MSA.

Cardiovascular and catecholamine responses to intravenously administered acetylcholine are being studied in patients with autonomic failure to look for evidence of ganglionic supersensitivity. In normal subjects, the plasma norepinephrine response is proportional to the logarithm of the acetylcholine infusion rate. PAF patients did not increase their plasma norepinephrine levels while patients with MSA responded only at the highest tolerated dose. Both MSA and PAF patients had exaggerated bronchoconstrictive responses to acetylcholine, suggesting parasympathetic supersensitivity to muscarinic stimulation. Unfortunately, this side effect limited the maximum dose of drug tolerated by the patients. Since this effect can be prevented by pretreatment with muscarinic antagonists in experimental animals, additional studies are planned which will include administration of glycopyrrolate. Although the preliminary results are consistent with post-ganglionic dysfunction in PAF and MSA, it is necessary to employ higher doses of acetylcholine to construct dose-response curves that can discriminate the responses among the subjects. Measurement of FEV₁ during acetylcholine infusion without glycopyrrolate pretreatment may also allow quantitative comparison of bronchoconstriction.

We have applied our previously developed biochemical and pharmacological methods to assess sympathetic neuronal function and neurotransmitter metabolism in two other disorders: narcolepsy and hypothyroidism. Some patients with narcolepsy and cataplexy have elevated basal levels of plasma norepinephrine. By using a self-administered rating scale for cataplexy, we have correlated the degree of plasma norepinephrine elevation with severity of cataplexy in these patients. Blood pressure responses to infused norepinephrine were normal suggesting that the increased basal norepinephrine levels are not compensatory for decreased vascular noradrenergic responsivity. Since narcolepsy is an inherited disorder and cataplexy is more likely the result of a central nervous system dysfunction, we are now examining CSF neurotransmitter metabolites to determine whether the elevated plasma norepinephrine levels are part of a genetically determined abnormality in neurotransmission. An HLA study is also in progress to confirm the association with HLA-B7 and DR2. It may be possible to separate patients with narcolepsy according to genetic predisposition, clinical features, and neuropharmacologic characteristics.

Hypothyroidism is attended by elevated plasma norepinephrine levels and downregulation of noradrenergic receptors; some patients also develop reversible orthostatic hypotension although catecholamines have not been measured in these patients. Our preliminary results in a group of patients who have undergone total thyroidectomy indicate that blood pressures do not differ between the hypo- and euthyroid states. Plasma catecholamine levels have not yet been analyzed.

Orthostatic hypotension has generally been treated by using a combination of fludrocortisone and ibuprofen. However, some patients do not respond well to this regimen. Two oral drug trials for management of orthostatic hypotension have been initiated. Minoxidil is being used because it may reduce the supine hypertension in patients with autonomic failure. It is expected to improve the orthostatic hypotension through reflex sympathetic activation and fluid retention. Minoxidil lowers blood pressure only into the normal range and has not been known to produce orthostatic hypotension. Although our initial experience with midodrine was not favorable due to the development of undesirable side effects, we are re-evaluating this drug as part of a multicenter controlled trial. Midodrine is a long-acting alpha agonist drug which contains a glycine residue that must be enzymatically cleaved to release the active moiety. Our sympathetic neural prosthesis will also be further refined into a treatment modality for patients who fail conventional management for orthostatic hypotension.

A number of additional studies are currently in progress:

- 1) Cardiovascular, catecholamine, and hormonal responses are being studied in response to intravenous administration of isoproterenol. We have previously shown that PAF patients have alpha-adrenergic receptor supersensitivity. In addition, gastric responses during insulin-hypoglycemia in PAF suggest that a similar sensitivity may occur in the beta-adrenergic system.
- 2) A detailed study of nocturnal polysomnography in MSA patients will characterize the changes in respiratory pattern, oxygen saturation and autonomic function that occur during sleep. Sleep apnea in MSA patients may be either central or obstructive. Since these patients commonly die during the night this phenomenon may play a role in the terminal event that shortens their lifespan.
- 3) A preliminary analysis of clinical and genetic aspects of autonomic failure in 70 patients has been completed. Although there is no obvious hereditary pattern, some families manifest a clustering of other central nervous system disorders. Our HLA study does not show any association in contrast to the increased incidence of AW32 in MSA reported by others.
- 4) A study of neuropathological changes in autonomic failure has confirmed the diagnoses in individual cases and revealed findings similar to those previously reported. An examination of our first autopsied case of PAF will soon be completed.
- 5) Detailed analysis of cerebral glucose metabolism in PAF and MSA using PET scanning is in progress.

The Clinical Neuropharmacology Section has continued the study of familial Alzheimer's disease as a major priority within the scope of its research efforts. Alzheimer's disease is a major medical and social problem since it is the most common cause of irreversible, chronic dementia. Clinical and therapeutic research of AD is significantly limited by both accuracy and

timing of diagnosis. More than 30% of clinically diagnosed cases do not have AD at autopsy. Although AD may be familial in only about a third of all cases, the main justification for studying the autosomal dominant subgroup of familial cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members. There are two major directions of our AD research: 1) to investigate genetic linkage in order to identify the primary molecular event underlying AD in these families, and 2) to define the clinical and biochemical progression of the disease through a longitudinal investigation of affected and at-risk subjects. The ultimate goal of our work is to provide clues for earlier, accurate diagnosis and more rational approaches to treatment of AD.

The intra-agency agreement between the NINCDS and NIA has facilitated the establishment of skin fibroblast and peripheral blood lymphoblast cultures at the Coriell Institute for Medical Research, Camden, New Jersey. More than 150 individuals from 5 pedigrees with autosomal dominant inheritance have been examined and biopsied. We are continuing to search for new branches of the families to make the pedigrees more informative from the standpoint of genetic studies. In particular, a collaborative effort has identified English ancestors of the Canadian family which may help to locate living descendants for our genetic linkage work. The cultures have served as a renewable source of DNA and cells for basic research in AD.

Although many candidate genes have been excluded as primary genes causing AD, our genetic linkage studies using restriction fragment length polymorphisms for chromosome 21 have identified a region near the centromere which shows positive linkage in 4 families included in the multi-point analysis. Of interest is the fact that the gene for amyloid has been cloned and found to reside within the region linked to AD. However, the amyloid marker does not give a significant low score in the families. Work is in progress to develop additional markers in this region. The combination of more highly polymorphic probes and additional family members should permit identification of a single marker for AD.

The longitudinal investigations have continued and psychological support and genetic counseling have been given. CSF levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), corrected for the plasma contribution, and somatostatin are reduced in AD patients compared to controls. The levels of homovanillic acid and 5-hydroxyindoleacetic acid are normal. These data suggest a selective involvement of the brain noradrenergic and somatostatinergic systems in dominantly inherited AD. Approximately half of the at-risk subjects had a positive rating according to the Blessed Dementia Scale. In these subjects there was a positive inverse correlation between the clinical rating and the level of corrected MHPG or somatostatin in the CSF. Although further follow-up will be required to determine which at-risk subjects develop dementia, these results indicate that changes in these neurotransmitter systems may precede clinical expression of AD.

Neuropathological investigation of the hippocampus has been completed on eight cases from three pedigrees with dominantly inherited AD. Regional plaque and tangle density markedly varied with CA 1-2 having the highest density of both.

Presubiculum contained virtually no plaques or tangles. Plaque size varied among regions with the largest senile plaques in CA4. However, statistical analysis disclosed no differences among the families in plaque density, distribution or size. These results show consistent patterns of hippocampal pathology, the distribution and characteristics of which appear to be determined in some manner by the hippocampal region in inherited AD.

Additional studies that have been continued or in progress include:

- 1) Examination of cerebral glucose metabolism in AD patients parallels the results reported in sporadic cases. No consistent pre-symptomatic changes have been observed in at-risk subjects.
- 2) Dermatoglyphic patterns are being analyzed in patients and their first-degree at-risk relatives.
- 3) DNA repair in at-risk subjects is being examined since a defect was previously observed in affected patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02318-10 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: William Theodore, M.D.

Acting Chief, CES

MNB

NINCDS

Other: Susumu Sato, M.D.

CES

MNB

NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒

(a) Human subjects

☐

(b) Human tissues

☐

(c) Neither

☒

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical Epilepsy Section has begun to place more emphasis on the clinical pharmacology of new antiepileptic drugs, with somewhat diminished studies of commonly used medical therapies. Pilot studies are now complete on felbamate (2-phenyl 1-1,3 propanediol dicarbamate), and the randomized placebo-controlled double-blind study was initiated. The purpose of this study is to obtain definitive information regarding the efficacy of felbamate in patients with uncontrolled partial seizures. The unique three period crossover design allows unbiased estimates of drug effects even in the presence of a carry-over effect from one period to the next. In a separate study of patients with partial seizures, a pilot effort will be undertaken to evaluate a very old compound, dapsone, for its effect on control of partial seizures. This anti-leprosy drug recently demonstrated efficacy in animal models--such as the maximal electroshock--and its protective index is promising. The study will evaluate the pharmacology of the drug in humans, identify drug interactions with current antiepileptic drugs, establish the maximum tolerated dose, and obtain preliminary assessment of efficacy of the drug. This study will be carried out in both inpatient and outpatient facilities, but with outpatient emphasis. Such will permit a long-term follow up of the possible usefulness of this drug in patients with partial seizures. A study of carbamazepine and its metabolites is continuing; an understanding of the major metabolite, carbamazepine 10-11 epoxide, is sought.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02236-12 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William Theodore, M.D.	Acting Chief, CES	MNB	NINCDS
Others: Susumu Sato, M.D.	CES	MNB	NINCDS
Mark Hallett, M.D.	OCD	MNB	NINCDS

COOPERATING UNITS (if any)

Office of the Clinical Director, NINCDS

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither
☒ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical Epilepsy Section has been developing and testing new techniques to improve seizure control, medication tolerance, and rehabilitation in patients with severe epilepsy. Patients with uncontrolled seizures are admitted for a complete evaluation, including simultaneous video and telemetered EEG recording of seizures, daily determinations of antiepileptic drug serum concentrations, positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetoencephalography (MEG). A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy. CSF biochemistry, and neurochemistry of temporal lobe specimens resected from patients with uncontrolled seizures, are being studied. PET in patients with localized brain lesions has demonstrated focal hypometabolic cerebral areas corresponding to the interictal seizure EEG focus. In some patients, PET has been able to detect a focus when other methods have failed. Studies of patients during partial seizures have shown a change from hypo- to hypermetabolism at the site of the focus. In the Lennox-Gastaut syndrome, PET has revealed the existence of two separate metabolic patterns despite clinical seizure similarity. PET has shown that antiepileptic drugs reduce cerebral glucose utilization. Phenobarbital decreases glucose utilization by 30-40%, while phenytoin and carbamazepine reduce metabolic rates by only 10-15%. This difference may be related to differences among the drugs in effects on cognitive function. PET studies allow more definitive identification of the epileptic lesion and suggest new avenues of investigation into the basic mechanisms of the epilepsies. MRI may show small structural lesions underlying PET hypometabolism even when CT is normal. Further studies will elucidate the relation of metabolic and pathologic changes. MEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the spatial distribution of epileptiform in cortical depths; MEG may be superior.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02562-05MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurophysiological Bases of Phonation and Voice Disorders*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Professional Personnel:

Christy L. Ludlow, Ph.D.	Speech Pathologist	HMCS, MNB, NINCDS
Susan E. Sedory, M.A.	Speech Pathologist	HMCS, MNB, NINCDS
Ralph R. Naunton, M.D.	Otolaryngologist	DCD, NINCDS
Mihoko Daito, M.D.	Visiting Fellow	HMCS, MNB, NINCDS
Geralyn Schulz, M.A.	Speech Pathologist	HMCS, MNB, NINCDS

COOPERATING UNITS (if any)

DCD, NINCDS

LAB/BRANCH

Medical Neurology Branch

SECTION

Human Motor Control

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.75

PROFESSIONAL:

.75

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose is to determine the neurophysiological control of phonation and the pathophysiology of voice disorders such as spasmodic dysphonia. The normal timing and different motor control functions (respiration, effort closure, swallow, phonation and speech) were examined. For speech, the muscles were independently activated at different times and the activation pattern was more precisely controlled than during swallow or phonation. The intra-subject muscle relaxant effects of diazepam found on the laryngeal muscles in all normal subjects studied thus far have been independent from diazepam effects on muscle activation patterns for speech and swallow. Consistent decreases in the percent activation for speech and swallow occurred in some subjects and significant increases in others. These individual differences may relate to a subject's age.

Patients with spasmodic dysphonia who have had unilateral recurrent laryngeal nerve resection are being studied neurophysiologically. Although all have vocal fold paralysis, close to normal activation levels have been found in the thyroarytenoid muscles on the resectioned side. Symptoms were reduced both by a temporary recurrent nerve block reducing muscle activation on the resectioned side and by botulinum toxin injection into the thyroarytenoid muscle on the same side, suggesting reinnervation following surgery. Nerve block and stimulation studies are being conducted in these patients to evaluate this possibility. Studies of the effects of acoustic reflexes, nerve stimulation and sensory nerve block on laryngeal muscle activation, have been initiated in patients with spasmodic dysphonia and normal volunteers.

*(Formerly: "Neurophysiological Bases of Phonatory Pathology")

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02563-05MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Independent Aspects of Speech Production in Neurological Disorders *

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.: Christy Ludlow, Ph.D. Speech Pathologist SPU, HMCS, MNB, NINCDS

Others: Celia J. Bassich, M.A. Speech Pathologist SPU, HMCS, MNB, NINCDS

Geraldyn M. Schulz, M.A. Speech Pathologist SPU, HMCS, MNB, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Medical Neurology Branch

SECTION

Human Motor Control

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.75

PROFESSIONAL:

.75

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two groups of patients with speech dyspraxia 15 years following penetrating head injury were compared on acoustic measures of the temporal organization of their speech during tasks requiring rate and stress changes. One group had a residual non-fluent aphasia with speech dyspraxia while the other had normal language functioning and dyspraxia. Although speech rate was significantly slowed in both groups, only those with a co-existing language disorder did not maintain a normal temporal organization of their speech during rate and stress changes.

Much of speech production research has employed nonsense syllables which can be phonetically controlled. Ongoing experiments are examining whether the regularity of production (speech invariance) and speech planning differs between highly familiar and unfamiliar words and nonsense syllables. These experiments involve normal controls, Parkinson patients and chronic adult stutterers. The development of a classification system for chronic adult stutterers has been initiated. Our aim is to relate the different speech characteristics seen in this group to a subclassification system which is predictive of treatment response.

Studies of the pathophysiology of oral-lingual dyskinesia before and after treatment are continuing.

*(Formerly: "Independent Aspects of Speech Timing in Neurological Disorders")

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 N5 02564-05 MN8

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Organization for Speech Production and Speech Perception*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Christy Ludlow, Ph.D. Speech Pathologist SPU, HMCS, MNB, NINCDS

OTHERS: Andres Salazar, M.D. Neurologist VHIS, Walter Reed Army Medical Center

Karen Schwab, M.S., Statistician VHIS, Walter Reed Army Medical Center

COOPERATING UNITS (if any)

VHIS, Walter Reed Army Medical Center, Washington, D.C.

LAB/BRANCH

Medical Neurology Branch

SECTION

Human Motor Control

INSTITUTE AND LOCATION

NINCDS, Bethesda, MD 20892,

TOTAL MAN-YEARS:

.25

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose is to determine the brain organization and inter-dependence between speech production, speech perception and language in adults. CT scan data from the Vietnam Head Injury Study provide information on lesion topography following penetrating missile wounds. These studies continue to demonstrate the importance of subcortical white matter tracts in the left hemisphere for speech and language recovery. Recovery patterns suggest considerable plasticity when lesions only involve the cortical speech and language areas. Some speech functions, such as rhythm and rate, are affected by unilateral lesions in subcortical structures of the left or right hemispheres. Therefore, the neurological control of some aspects of speech production are separate from language. Similarly, in speech perception, only the perception of the temporal order of speech sounds was associated with lesions in the left hemisphere but the identification of rapid stop gaps was not. Positron emission tomography is being conducted in normal volunteers after performing a psychophysical test of temporal order perception for speech sounds. The aim is to determine whether performance is related to increased metabolism in the left temporal region.

*[Formerly: "Relationships Between Language and Speech Deficits in Neuropathologies"]

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02667-03 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Involuntary Movements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director		OCD	ODIR	DIR	NINCDS
		Chief	MDU	HMCS	MNB	DIR	NINCDS
Others:	Richard Dubinsky, M.D.	Medical Staff Fellow	MDU	HMCS	ODIR	DIR	NINCDS
	Bruce Geller, M.D.	Medical Staff Fellow		OCD	ODIR	DIR	NINCDS
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	DIR	NINCDS
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	DIR	NINCDS
	Robert Spitzer, M.D.	Medical Staff Fellow	MDU	HMCS	MNB	DIR	NINCDS

COOPERATING UNITS (if any)

Department of Nuclear Medicine, Clinical Center

LAB/BRANCH

Medical Neurology Branch, DIR

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

1.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Myoclonus and a number of other rapid involuntary movements have been difficult to classify clinically. Clinical and physiological analysis of a continuing series of patients has led to new classifications and pathophysiological insights. A series of patients with epilepsia partialis continua have been studied; the pathophysiology is heterogeneous, but in most electrophysiological techniques can identify an excitable region in cerebral cortex. Positron emission tomography (PET) studies of patients with palatal myoclonus have revealed that the inferior olives are hyperactive. PET studies of patients with hemiballismus have revealed that hypoactivity of the ipsilateral striatum occurs as a result of the lesion in the subthalamic nucleus. PET studies of patients with essential tremor have revealed that the inferior olives are hypermetabolic during the tremor, but not when the patients are at rest.

In studies of postural action tremors, we have been analyzing the amplitude of the tremor as a function of the precise posture of the limbs. For many tremors, such as cerebellar postural tremor, the tremor is worse when the arms are near the body and the hands are pointing towards each other. For other tremors this is not true, and the clinical and physiological significance of this is being explored.

Task specific focal dystonias of the hands such as writer's cramp and pianist's cramp have been analyzed and a number of physiological characteristics have been defined. There appears to be diminished ability to control the fingers independently and gating of somatosensory evoked potentials with voluntary movement is abnormal. The spasms themselves have been characterized into different patterns. Abnormalities of the blink reflex have been identified in dystonic disorders. We have verified this in a number of our own patients and are now applying this test to the patients with focal hand cramps. These patients have also been studied with a specific test for analysis of reciprocal inhibition in the arm. Reciprocal inhibition is diminished in the arm which shows the focal dystonia, but not in the arm which is not dystonic.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02668-03 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trial of Isoniazid for Action Tremor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	DIR	NINCDS
		Chief	MDU	HMCS	MNB	DIR NINCDS
Others:	Richard Dubinsky, M.D	Medical Staff Fellow	MDU	HMCS	ODIR	DIR NINCDS
	Bruce Geller, M.D	Medical Staff Fellow		OCD	ODIR	DIR NINCDS
	Jerome Sanes, Ph.D	Senior Staff Fellow	MDU	HMCS	MNB	DIR NINCDS
	Leo Cohen, M.D	Visiting Associate	MDU	HMCS	MNB	DIR NINCDS
	Robert Spitzer, M.D	Medical Staff Fellow	MDU	HMCS	MNB	DIR NINCDS

COOPERATING UNITS (if any)

None

LAB BRANCH

Medical Neurology Branch, DIR

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies have shown utility of isoniazid for ameliorating cerebellar postural tremor in patients with multiple sclerosis. Current studies are aimed at identifying whether patients with action tremors of other types are also benefitted. A double-blind placebo-controlled, cross-over trial is in progress. Fifteen patients have now been studied. Isoniazid does appear to help some of these cases, but we do not have enough experience yet to predict the response.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02669-03 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Voluntary Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	DIR	NINCDS
		Chief	MDU	HMCS	MNB	DIR NINCDS
Others:	Richard Dubinsky, M.D.	Medical Staff Fellow	MDU	HMCS	ODIR	DIR NINCDS
	Bruce Geller, M.D.	Medical Staff Fellow		OCD	ODIR	DIR NINCDS
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	DIR NINCDS
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	DIR NINCDS
	Robert Spitzer, M.D.	Medical Staff Fellow	MDU	HMCS	MNB	DIR NINCDS

COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center
 Department of Nuclear Medicine, Clinical Center

LAB/BRANCH

Medical Neurology Branch, DIR

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in the Gait Laboratory of the Department of Rehabilitation Medicine have focussed on the control of balance. Simultaneous measurement of body angles, foot-floor forces and multiple EMGs are possible. Studies in normal subjects have revealed insights into the biomechanical effects of postural muscle activity. Similar studies have been initiated in patients with cerebellar disturbance and Parkinson's Disease.

In patients with Parkinson's Disease there was a positive correlation between circulating levels of dopamine and cognitive motor capabilities in a choice reaction time situation but not in a simple reaction time situation. In normal subjects, triggered muscle responses produced by stopping voluntary movements were similar to muscle responses generated when subjects initiated a voluntary movement from the stopped location, thereby suggesting that triggered and voluntary reactions are mediated by similar central nervous system mechanisms.

The study of motor control in hemiplegia is being planned and apparatus prepared. Patients with discrete brain lesions will be studied; patients with strokes will be the main group, and many patients will be followed serially from onset of the disorder to recovery. Preliminary studies with positron emission tomography (PET) of normal subjects have shown that it is difficult to identify cortical areas active with voluntary movement using fluoro-deoxy-glucose (FDG) as the tracer. Further studies are planned with oxygen-15-water which will probe blood flow rather than glucose metabolism.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02711-02 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	DIR	NINCDS	
		Chief	MDU	HMCS	MNB	DIR	NINCDS
Others:	Bruce Geller, M.D.	Medical Staff Fellow	OCD	ODIR	DIR	NINCDS	
	Richard Dubinsky, M.D.	Medical Staff Fellow	MDU	HMCS	ODIR	DIR	NINCDS
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	DIR	NINCDS
	Christy Ludlow, Ph.D.	Speech Pathologist	SPU	HMCS	MNB	DIR	NINCDS
	Ralph Naunton, M.D.	Otolaryngologist		CDP	EAP	NINCDS	

COOPERATING UNITS (if any)

None

LAB BRANCH

Medical Neurology Branch, DIR

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Botulinum toxin injected in small doses directly into muscle binds to the neuromuscular junction and inactivates it for approximately 3 months. This treatment has been demonstrated to be useful for strabismus and blepharospasm, but there has not been a complete understanding of its mechanism of action.

Studies of utility of botulinum toxin have been carried out in spasmodic dysphonia and writer's cramp (and its variants such as pianist's cramp). Treatment appears efficacious for both. Further studies are in progress with adult stuttering.

Studies of physiology of the mode of action have been carried out in spasmodic dysphonia, writer's cramp, blepharospasm and hemifacial spasm. These studies show that the major effect of botulinum toxin is to weaken the muscle that is in spasm. EMG studies show that spasms continue, but muscles are ineffective. No other changes in physiology can be identified.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02712-02 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Non-invasive Stimulation of Human Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	DIR	NINCDS
		Chief	MDU	HMCS	MNB	DIR NINCDS
Others:	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	DIR NINCDS
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	DIR NINCDS

COOPERATING UNITS (if any)

None

LAB BRANCH

Medical Neurology Branch, DIR

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.3

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recently techniques have become available for the non-invasive stimulation of the human cortex and human spinal cord. A device has been manufactured which produces a high voltage, extremely brief pulse which can penetrate skull and activate central nervous system without excessive pain. Previous studies have shown it useful in some circumstances where evaluation of the speed and integrity of motor pathways is valuable.

Our project to map the motor cortex has demonstrated that it is possible to identify discrete areas for the leg, hand, upper arm and face. Studies with somatosensory evoked potentials have shown that the motor areas are discrete from, and anterior to, the primary sensory areas as revealed by the topography of the early components.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02630-04 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CLINICAL, GENETIC, AND BIOCHEMICAL STUDIES OF FAMILIAL ALZHEIMER'S DISEASE

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R. J. POLINSKY, M.D.	CHIEF	CNS, MNB, NINCDS
OTHERS:	L. E. NEE, M.S.W.	SOCIAL SCIENCE ANALYST	OCD, NINCDS
	R. T. BROWN, M.D.	MEDICAL STAFF FELLOW	CNS, MNB, NINCDS
	G. DI CHIRO, M.D.	CHIEF	NIS, OD, DIR, NINCDS
	J. GRAFMAN, Ph.D.	PSYCHOLOGIST	CLPS, MNB, NINCDS

COOPERATING UNITS (if any)

LAB. OF HIS TOPATH., LA SALPETRIERE (J. FONCIN); GENETICS UNIT, DEPT. OF NEUROLOGY, MASS. GEN. HOSP. (J. GUSELLA, P. HYSLOP); DEPT. OF GENETICS, INDIANA UNIV. (M. CONNEALLY); NEUROPATH. LAB., JOHNS HOPKINS HOSP. (D. PRICE, R. STRUBLE); DEPT. OF NEUROLOGY, UNIV. OF VERMONT (S. ROBISON, W. BRADLEY)

LAB/BRANCH

MEDICAL NEUROLOGY BRANCH, DIR, NINCDS

SECTION

CLINICAL NEUROPHARMACOLOGY SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, BETHESDA, MD 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alzheimer's disease is the most common cause of irreversible, chronic dementia. Although AD may be familial in only one third of all cases, the main justification for studying autosomal dominant cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members. Neuropathological investigation of the hippocampus has been completed on eight cases from three pedigrees with dominantly inherited AD. There was regional variability in plaque and tangle density as well as plaque size but no significant differences were observed among the families. Previous genetic studies have not clarified the role of inheritance. More than 150 members of 5 pedigrees with an autosomal dominant form of AD have had cultures established from skin fibroblasts and peripheral blood lymphoblasts. These cultures serve as a renewable source of DNA and cell lines for genetic linkage, viability, and biochemical studies. Recombinant DNA technology has been applied to genetic linkage studies in 4 large pedigrees with inherited AD. Many candidate genes have been excluded from linkage with AD. Using several restriction fragment length polymorphisms and a multi-point linkage analysis it has been shown that the genetic defect causing Alzheimer's disease maps to chromosome 21. A longitudinal investigation of affected and at-risk subjects is being conducted. CSF levels of neurotransmitter metabolites and neuropeptides reveal a selective involvement of brain noradrenergic and somatostatinergic systems in inherited AD. Neurochemical changes in at-risk subjects may precede clinical expression of the disease. Although cerebral glucose metabolism is reduced in AD patients, no consistent changes have been observed in asymptomatic at-risk subjects.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02115- 14 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

BIOCHEMICAL INDICES OF ADRENERGIC FUNCTION IN HUMANS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: R. J. POLINSKY, M.D. CHIEF CNS, MNB, NINCDS

OTHERS: R.T. BROWN, M.D. MEDICAL STAFF FELLOW CNS, MNB, NINCDS
 L. E. NEE, M.S.W. SOCIAL SCIENCE ANALYST OCD, NINCDS
 I. J. KOPIN, M.D. CHIEF NPS, LNDR, NINCDS

COOPERATING UNITS (if any)

DIVISION OF ENDOCRINOLOGY, VA HOSPITAL, WASHINGTON, DC (L. RECENT); NEUROPATHOLOGY
 LAB, JOHNS HOPKINS HOSP., BALTIMORE, MD (D. PRICE, J. TRONCOSA)

LAB/BRANCH

MEDICAL NEUROLOGY BRANCH, DIR, NINCDS

SECTION

CLINICAL NEUROPHARMACOLOGY SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, BETHESDA, MD 20892

TOTAL MAN-YEARS:	3.0	PROFESSIONAL:	2.0	OTHER:	1.0
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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Autonomic nervous system activity is essential for maintaining circulatory and metabolic homeostasis. In order to study sympathetic nervous system function and its relationship to other neuroendocrine systems, it is necessary to measure neurotransmitter, hormonal, and peptide levels in response to various stimuli. The levels of norepinephrine, epinephrine, and dopamine and their metabolites in various body fluids reflect the activity of the neurones from which these neurotransmitters are released. Cerebrospinal fluid levels of monoamine metabolites can be used to assess central nervous system neurotransmitter metabolism. It is necessary to consider the origin of these metabolites to make appropriate corrections for valid interpretations of the data. These strategies have been used to study patients with neurogenic orthostatic hypotension and other disorders in which abnormal adrenergic function is suspected. Insulin may play a role in causing post-prandial hypotension in patients with autonomic dysfunction but the mechanisms may differ in pure autonomic failure (PAF) and multiple system atrophy (MSA). Glucose partially prevents insulin-induced hypotension in MSA but not PAF. An hypoglycemic mechanism involving endorphins may also be involved in MSA since naloxone reduces the blood pressure decrement during hypoglycemia. Preliminary results suggest that beta-endorphin responses to arecoline, a muscarinic agonist, are deficient in MSA. Ganglionic sensitivity to acetylcholine in normal subjects is manifested by a semilogarithmic relationship between plasma norepinephrine levels and drug dose. Exaggerated bronchoconstrictive responses in PAF and MSA suggest parasympathetic supersensitivity. Investigations of autonomic function in aging, narcolepsy, and hypothyroidism are in progress. Elevated plasma norepinephrine levels correlate with severity of cataplexy in patients with narcolepsy. A more thorough understanding of neurotransmitter metabolism in these clinical situations leads to more rational approaches to therapy. Several drug trials for managing orthostatic hypotension including further refinement of a sympathetic neural prosthesis are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01658-20 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Hemispheric Development and Specialization of the Intellectual Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Psychologist	MN	NINCDS
	J. Grafman, Ph.D.	Psychologist	MN	NINCDS
Others:	C. Cox, M.S.	Psychologist	MN	NINCDS
	C. Kufita, M.D.	Medical Officer	SN	NINCDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINCDS

LAB/BRANCH

Medical Neurology, DIR, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The disabling effects of chronic cerebral insult and neuropsychiatric disorders were evaluated by a broad range of neuropsychological tests evaluating brain-behavior relations in man.

Asymptomatic long term survivors of acute lymphoblastic leukemia (ALL) who received CNS preventive therapy (cranial irradiation and intrathecal chemotherapy) were studied. Based on CT scan findings, the patients were divided into three groups: normal scans, cortical atrophy; intracerebral calcifications. Memory and learning were significantly impaired in children with abnormal scans, more so for the patients with calcification. In addition, all patients with abnormal CT scans showed significant attentional dysfunctions.

Adolescents with obsessive compulsive features exhibited a cluster of neuropsychological deficits which correlated with ventricular enlargement. Deficits were identified in spatial judgment and spatial learning. It was suggested that an imbalance in the inhibitory functions of the frontal lobe and limbic systems may contribute to obsessive compulsive behavior.

Patients with Tourette's Syndrome were evaluated in an effort to characterize the neuropsychological defects in frontal inhibitory mechanisms. Positron emission tomographic (PET) data of regional cerebral activity from these patients will be correlated with test performance.

35-MNB/DIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01424-21 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Modulation by the Limbic System in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Psychologist	MN	NINCDS
	J. Grafman, Ph.D.	Psychologist	MN	NINCDS
Others:	C. Cox, M.S.	Psychologist	MN	NINCDS
	F. Lalonde, M.A.	Psychologist	MN	NINCDS
	E. Mohr, Ph.D.	Psychologist	MN	NINCDS
	E. Witt, Ph.D.	Psychologist	MN	NINCDS
	C. Kufita, M.D.	Medical Officer	SN	NINCDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINCDS

LAB/BRANCH

Medical Neurology, DIR, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Emotional and cognitive characteristics were studied in epileptic patients before and following unilateral left or right temporal lobe resection. The integrity of attentional and perceptual (visual, auditory, and tactile) systems were evaluated using standard and experimental procedures. Physiological events (skin conductance) were also monitored during test performance. The research examined the role of the temporal lobe in establishing specific limbic associations between left and right hemispheres in regulating cognitive functions and emotional experiences in man.

Tachistoscopic studies identified a critical perceptual role for right temporal mechanisms, especially during initial visual processing. The left and right temporal lobes contribute differentially to specifying the identity of a stimulus and its position or orientation in space. Left temporal mechanisms encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategies which combine overt and covert imagery with praxic encoding.

In affective sectors, left temporal patients tend to neutralize reactions to nuances with emotional coloration; right temporal patients, in contrast, rate these materials similar to normal individuals. Unlike normal individuals, however, the left and right temporal lobectomy patients were hyporesponsive to affective material as indexed by skin conductance measures. Moreover, both lobectomy groups failed to take advantage of the emotional characteristics of information to facilitate memory. These data suggest that unilateral temporal lobectomy disrupts the normal linkage of cognitive-affective associations mediated by temporal limbic interaction. There were, however, beneficial effects to surgical treatment in that patients, following temporal lobe surgery, were less deviant from normal subjects in emotional behavior.

36-MNB/DIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01245-22 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. Johnson, Jr., Ph.D.	Psychologist	MN	NINCDS
	P. Fedio, Ph.D.	Psychologist	MN	NINCDS
Others:	J. Grafman, Ph.D.	Psychologist	MN	NINCDS
	C. Kufta, M.D.	Medical Officer	SN	NINCDS
	R. Porter, M.D. (departed 5/87)	Medical Officer	MN	NINCDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINCDS

LAB/BRANCH

Medical Neurology, DIR, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Information processing was monitored by averaged evoked response techniques. The electrographic activity was recorded from left and right brain regions during memory and perception in normal subjects, patients with unilateral temporal lobectomy, and patients with neuropsychiatric disorders. Electroencephalographic disturbances in brain-behavior relations in psychiatric patients were also evaluated, relating left and right brain dysfunctioning to activity to maladaptive ideative and emotional reactions, respectively.

In temporal lobectomy patients, P300 amplitude was found to be inversely proportional to stimulus probability in the same way as for normal controls, and larger P300s were elicited in reaction time. For visual material, right-temporal patients manifested smaller P300s at frontal sites than either left-temporal or normal individuals. Moreover, there were no consistent hemispheric asymmetries which distinguished the left-or right-temporal patients, or either group from normal subjects. These data discount the hypothesis that medial temporal structures, including the hippocampus, serve as a sole generator of P300. More specifically the data indicate that processing of auditory and visual stimuli is dependent to a great extent on the character of the material and the integrity of left and right brain mechanisms.

37-MNB/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 00200-33 MNB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cognitive and Emotional Profile of Neuropsychiatric Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	P. Fedio, Ph.D.	Psychologist MN NINCDS
	E. Mohr, Ph.D.	Psychologist MN NINCDS
Others:	C. Cox, M.S.	Psychologist MN NINCDS
	J. Grafman, Ph.D.	Psychologist MN NINCDS
	F. Lalonde, M.A.	Psychologist MN NINCDS
	E. Witt, Ph.D.	Psychologist MN NINCDS
	T. Chase, M.D.	Neurologist ET NINCDS
COOPERATING UNITS (if any) Experimental Therapeutics Branch, DIR, NINCDS		
LAB/BRANCH Medical Neurology, DIR, NINCDS		
SECTION Clinical Neuropsychology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A neuropsychological profile of <u>dementia</u> was drafted for individuals with <u>Alzheimer's Disease</u>, <u>Huntington's Disease</u> and '<u>at risk</u>' for <u>Huntington's Disease</u>. The evaluations extended into <u>memory</u>, <u>learning</u> and <u>perceptual</u> areas, utilizing standard and experimental tasks, also establishing normative references for functional changes accompanying the aging processes. Although Alzheimer's Disease is accompanied by marked deficits in selective attention, memory and learning, there were no qualitative differences between demented and age-matched subjects. The impairment also extended to object-naming and fluency, and AD patients performed poorly in perceiving meaning, except when the stimuli required emotional judgment. The data indicate that Alzheimer's patients may be unable to encode material; this is in sharp contrast with other amnesic disorders where the primary difficulty involves an inability to store and/or retrieve information. Alzheimer's and Huntington's patients showed pronounced but dissimilar deficits with visuospatial and constructional tasks. The behavioral data extend neuropathologic impressions of degeneration of the frontal striatal system in Huntington's Disease, and temporo-parietal, cortical involvement in Alzheimer's Disease. The neuropsychological test profile of Alzheimer's patients yielded different clinical subgroups or populations. Memory and learning deficits, per se, were poor indicators of group membership. One group was characterized by severely impaired verbal abilities, but with intact perceptual and constructional skills. The second group was more impaired on perceptuomotor than verbal tasks. The third group showed comparable deficiencies in both linguistic and visual spatial sectors. Positron emission tomographic and EEG data confirmed corresponding changes in left, right or bilateral regions in the posterior cerebral quadrant, respectively.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02678-03 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Involvement of Calcium, Fodrin, Glutamate, and Catecholamines in Seizures*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Suzan Nadi, Ph. D. Senior Staff Fellow NEXS MNB NINCDS

OTHERS: David Goldstein, M.D. HEB NHLBI
 Leah-Devlin- Capelotti Biologist NEXS MNB NINCDS
 Manuela Pintor, M.D. Visiting Associate NEXS MNB NINCDS
 Karen Wayns-Lee Bio Lab Tech NEXS MNB NINCDS

COOPERATING UNITS (if any)

University of Tennessee (A. Wyler); Yale University (D. Morrow); Hebrew University (D. Lichstein)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	0.2	PROFESSIONAL:	0.2	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The work will involve the study of epileptic foci and the evaluation of the status of glutamate receptors, the cytoskeletal protein fodrin and the flux of calcium ions. The epileptic seizure has been demonstrated to occur as a result of a shift of external calcium ions to the interior of the cell. Since calcium ions are also known to activate calpain, which degrades fodrin, which is responsible for maintaining the integrity of the membrane. The question of interest in this study is to determine whether fodrin breakdown is directly linked to the spread of epileptic activity.

In addition the epileptic focus obtained from patients with intractable seizures will be screened for abnormalities in amino acid, catecholamine and neuropeptide levels.

The major findings of the project can be summarized as follows:

- Fodrin has been found to degrade immediately following a seizure.
- In the human epileptic focus the amino acids aspartate, glutamate and glycine were elevated in the epileptic focus when compared to non focal tissue removed from the same patients. Gamma amino butyric acid was not found to be different between focus and non focus.
- The peptides somatostatin and neuropeptide Y were elevated in the focus. Other peptides such as endorphin, corticotropin releasing factor, vasoactive intestinal peptide were not altered significantly.
- The catecholamines dopamine, norepinephrine and dopa were all elevated in the epileptic focus.
- The enzymes tyrosine hydroxylase and choline acetyltransferase were elevated in the epileptic focus. Glutamic acid decarboxylase and Na-K ATPase showed no significant difference.
- The muscarinic cholinergic receptors the β and α receptors were lower, whereas NMDA receptors were higher in the epileptic focus.

39-MNB/DIR

* [Formerly "Involvement of Calcium, Fodrin, and Glutamate in Seizures"]

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02679-03 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Plasma and CSF Levels of Neurotransmitters in Epileptic and to EKG Correlation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Suzan Nadi, Ph. D Senior Staff Fellow NEXS MNB NINCDS

OTHERS: Orrin Devinsky, M D Medical Staff Fellow OCD MNB NINCDS
Karen Wayns-Lee Bio Lab Tech NEXS MNB NINCDS

COOPERATING UNITS (if any)

Rockefeller University (S. Gandy)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Blood and cerebrospinal fluid (CSF) samples will be obtained from patients with epilepsy before and after seizure. The sample will be stored frozen in aliquots, and assayed for peptides by radioimmunoassay, for amino acids by fluorescence, and for catecholamines by HPLC linked to an electrochemical detector. These findings will be correlated to any EEG or EKG changes which occur during seizures in order that some insight into the sudden death syndrome in epilepsy might be obtained.

The level of peptides somatostatin, neurotensin, substance P, β endorphin, enkephalin, bombesin, neuropeptide Y, prolactin, ACTH, corticotropin releasing factor, CCK were studied and no significant differences were found. (See Table 1).

The levels of 23 amino acids were also determined but no significant alterations were observed in pre- and postictal CSF (Table II).

The presence of an ouabain like factor was detected in the CSF. This factor was elevated in the CSF postictally. Furthermore a band of protein of molecular weight of 100,000 was found to be phosphorylated in the early postictal phase and lose its phosphorylated state in the late postictal phase.

Postictal levels of norepinephrine, HVA, SHIAA were all elevated in the CSF.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02713-02 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Involvement of Abnormalities in GAD DNA in the Kindled Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Suzan Nadi, Ph.D. Senior Staff Fellow NEXS MNB NINCDS

OTHERS: Joan Schwarz, Ph.D. Research Chemist NPS LNGR NINCDS

COOPERATING UNITS (if any)

University of Tennessee (A. Wyler); Surgical Neurology branch, NINCDS (C. Kufta); UCLA (A. Tobin)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In situ hybridization of glutamate decarboxylase (GAD) DNA will be analyzed in kindled rats to determine if any abnormalities in this enzyme exist. Hybridization techniques will be used to determine whether there are polymorphisms in or around the GAD gene in patients with absence seizures.

Molecular biologic techniques such as the study of restriction fragment length polymorphisms (RFLPs) have been used to investigate the genetic aspects of a number of neurologic diseases such as Huntington's Chorea and Alzheimer's disease. A number of epidemiological studies indicate that absence seizures (otherwise known as petitmal) may recur in families. These observations imply that this form of epilepsy may have a multifactorial inheritance pattern. In order to determine whether there is a DNA abnormality linked to this form of seizure. We plan to study RFLP using cDNA to different enzymes (for neurotransmitters such as glutamic acid decarboxylase) or neuropeptides. The source of the DNA assayed by standard restriction enzyme cut plus southern blot techniques will be transformed lymphoblasts from patients with absence seizures, their ill first degree relatives and normal neurologic controls. The cDNA's will be obtained from various laboratories around the country

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02431-08 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Epilepsy: Seizures Produced by Kindling in Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Shun-ichi Yamaguchi, Ph.D. Psychologist MNB, NINCDS

OTHERS: Susumu Sato, M.D. Medical Officer MNB, NINCDS
Stuart Walbridge Lab Specialist MNB, NINCDS

COOPERATING UNITS (if any)

Office of the Clinical Director, DIR, NINCDS

LAB. BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.6

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In rats, seizures produced by chronic stimulation (kindling) of amygdaloid complex or other nuclei were studied.

A. Amygdaloid and globus pallidus kindling lead to notably different clinical seizure patterns in rats. Local cerebral blood flow determination by [¹⁴C] antipyrine autoradiography indicated that globus pallidus kindling led to LCDF increases in mostly non-limbic structures, whereas amygdaloid kindling led to increases in mostly limbic structures.

B. Different parts of rat hippocampus may not be homogeneous in their functional properties. Kindling by ventral hippocampus stimulation proceeds at a greater rate than by dorsal hippocampal stimulations. Also, lesions in ventral hippocampus appear to facilitate initial kindling, if they are made prior to the kindling procedure. However, if ventral hippocampus is lesioned after initial kindling, the lesions abolish it. Furthermore, the rekindling is impossible or severely retarded.

C. Certain monoclonally raised antibody to a rat hippocampal protein inhibits long-term potentiation (LTP) effect. Since LTP is indicated to be involved in kindling, effects of injecting this antibody with lateral ventricle on kindling were studied. Preliminary indications are not contradictory to this expectation, but definitive trend was not yet established.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02735-01 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigations of ATPase and Ouabain-like Factor in Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Suzan Nadi, Ph. D. Senior Staff Fellow NEXS MNB NINCDS

OTHERS: Leah Delvin-Capelloti Biologist NEXS MNB NINCDS

COOPERATING UNITS (if any)

Hebrew University (D. Lichtstein); University of Tennessee (A. Wyler)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Na⁺-K⁺ ATPase and ouabain binding have been reported to be decreased in the kindled rat model of epilepsy. Na⁺-K⁺ ATPase is involved in maintaining the membrane potential at 65mV. A decrease in the activity of the enzyme will cause depolarization of the cell membrane. Our work will involve the investigation of the status of the Na⁺-K⁺ ATPase system in spiking and non spiking tissue from the human temporal lobe by measuring the activity of the enzyme as well as the binding of ouabain. An attempt will be made in these studies to demonstrate an alteration in the activity of the enzyme.

As can be noted in Table I Na⁺-K⁺ ATPase activity is not altered in the kindled rat brain. Table II indicates that ouabain binding is not altered in the human cortex. Such a lack of alteration may indicate that a ATPase abnormalities occur in a very small number of cells and cannot be detected with current procedures. Table III shows that the binding of ouabain is altered in the brain of the kindled rat.

To determine if the ATPase has a role in the epileptic tissue from animals and human brain,

kindled rats and human brain will be evaluated for ATPase activity as well as ouabain binding activity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02737-01 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transplant of GABAergic Neurons into the Substantia Nigra of Kindled Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Suzan Nadi, Ph.D Senior Staff Fellow NEXS MNB NINCDS

OTHERS: Estrada Bernard, M.D Medical Staff Fellow NEXS MNB NINCDS
M. Paola Canevini, M.D Visiting Associate NEXS MNB NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project will investigate the effect of transplanting neuronal suspensions from the cerebellum of 16 day old rat embryos into the substantia nigra of adult kindled rats. The hypothesis is that the substantia nigra is involved in the control of spread of seizures and the addition of gamma amino butyric acid (GABA) ergic neurons to the nigra will prevent the seizures.

GABA ergic neurons are obtained by gentle tyrosinization of the cerebellum of 16 days old embryos. These cells are then transplanted into the substantia nigra using a Harvard pump. The origin of the cells will be determined by staining with the glutamate decarboxylase (GAD) antibody as well as cerebellum specific antibodies.

Our most recent results show at a light microscopic level a fine network of cells at the area of transplant in the nigra. These cells are elongated in shape with extensive processes. They have failed to stain with glutamate decarboxylase (GAD) antibody, but other GABA ergic neurons in the area have not stained either. We are currently investigating the reasons behind this. Preliminary work has shown that kindling disappears for 2 weeks after transplant but then returns. The reasons for these findings are being investigated.

The substantia nigra has been shown to be a controlling point of seizure spread by numerous investigators. The current study will attempt to demonstrate whether transplants of GABA ergic neurons from hot embryo cerebellum will block the spread of seizures.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02736-01 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Kindled Seizure Suppression by Cysteamine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Suzan Nadi, Ph.D. Senior Staff Fellow NEXS MNB NINCDS

OTHERS: M. Paola Canevini, M.D. Visiting Associate NEXS MNB NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cysteamine has been shown to suppress kindled seizures at doses from 90 mg/kg to 300 mg/kg when given to rats kindled to stage V. This project will involve a systematic evaluation of the alterations in brain chemistry and the onset of the suppression of seizures in order to better understand the mechanism of action of cysteamine.

The aims of the present project are to better understand the mechanisms by which cysteamine eliminates seizures. Rats which are kindled and sham operated will receive a single intraperitoneal injection of cysteamine (200 mg/kg). Following the administration of the drug the animals will be observed for behavioral changes as well as seizure suppression. The rats will be killed at known time intervals following cysteamine administration, the brain removed, and the cortex, cerebellum, midbrain, pons-medulla, and hippocampus will be dissected. These tissues will be extracted and evaluated for peptide, amino acid, receptor, and catecholamine levels. The correlation of the chemical changes to the seizure suppression may allow the identification of one chemical alteration with a decrease in seizure activity. The next step in the study will be to determine if antagonists of the compound will also suppress seizures. Studies at present from our laboratory as well as others show that both somatostatin and norepinephrine levels are decreased as a result of cysteamine administration. Our current studies are aimed at dissociating the involvement of these two chemicals in the mechanism of cysteamine action using pharmacological methodologies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02732-01 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological Studies of Ion Channels in Cultured Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael Rogawski, M.D., Ph.D.	Senior Staff Fellow	NEXS	MNB	NINCDS
Others: Setsuo Suzuki, M.D.	Fogarty Fellow	NEXS	MNB	NINCDS
K. Inuoe, Ph.D.	Fogarty Fellow	FDA		
Jeffrey Barker, M.D.	Chief	LNP		NINCDS

COOPERATING UNITS (if any)

LAB BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ability of clinically relevant drugs to interact with voltage-dependent ion channels was studied in several excitable cell types using the whole cell patch clamp and single channel recording techniques. GH₃ cells, a clonal line derived from rat anterior pituitary, were used as a model system to study two distinct potassium currents: (1) a transient, rapidly activating and inactivating current that showed strong steady state inactivation (I_{TOC}) and (2) a slowly activating, non-inactivating calcium-dependent current [$I_{K(Ca)}$]. The macroscopic and single channel currents for both of these conductances were identified and studied in detail. Aminopyridines, a class of drugs with powerful convulsant activity and several clinical applications, at low concentrations specifically blocked I_{TOC} and at higher concentrations facilitated $I_{K(Ca)}$. In a separate study, tetrahydroaminoacridine, a drug that may provide functional improvement in Alzheimer's disease, was found to block voltage-dependent potassium channels in cultured rat hippocampal neurons. In addition to these studies on potassium channels, a novel calcium-dependent chloride current was identified and characterized in GH₃ cells. This conductance was found to be regulated by GTP analogs. Similar chloride currents in neurons may control repetitive discharges such as occurs during seizures.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02733-01 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitability Properties of Enzymatically Dissociated CNS Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Rogawski, M.D., Ph.D.	Senior Staff Fellow	NEXS	MNB	NINCDS
Other:	Setsuo Suzuki, M.D.	Fogarty Fellow	NEXS	MNB	NINCDS

COOPERATING UNITS (if any)

LAB BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|---|--------------------------------------|
| ☐ (a) Human subjects | ☒ (b) Human tissues | ☐ (c) Neither |
| ☐ (a1) Minors | | |
| ☐ (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the reporting period, a technique was developed for isolating neurons from adult mammalian brain by enzymatic dissociation of brain slices. These neurons were found to be suitable for whole cell current clamp and voltage clamp recording using the gigohm seal patch technique. Recordings from enzymatically dissociated neurons obtained from guinea pig hippocampus and thalamus (dorsal lateral geniculate nucleus) demonstrated that they were capable of firing action potentials and responding to neurotransmitters, including amino acids (GABA and glutamate) and biogenic amines (norepinephrine). The properties of several voltage-dependent ionic conductances were characterized. Studies in progress include (1) analysis of the ionic basis and intracellular signalling mechanisms of the transmitters, and (2) determination of the effects of clinically significant drugs on the ionic conductance systems.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02734-01 MNB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electrophysiological Recording from Brain Neurons During Epileptic Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Rogawski, M.D., Ph.D.	Senior Staff Fellow	NEXS	MNB	NINCDS
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COOPERATING UNITS (if any)

LAB BRANCH

Medical Neurology Branch, DIR, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

A technique has been developed for electrophysiological recording from single brain neurons during experimentally-induced epileptic seizures in the rat. During the reporting period, studies were carried out on norepinephrine (NE)-containing neurons in the locus coeruleus. Central NE neurons have been implicated in the regulation of seizure activity in several animal seizure models. These experiments were designed (1) to determine how NE neurons respond during seizures so as to better understand their modulatory role and (2) to understand the changes occurring in CNS NE systems as a result of seizures. Distinctive changes in the firing activity of locus coeruleus NE neurons were observed with chemically (pentylentetrazol) and electrically (electroshock) induced seizures. Both treatments induced intense firing in the perictal period with depression of firing or bursting in synchrony with cortical spiking after the major seizure discharge. These studies help to explain biochemical changes occurring in human epileptic brain, provide further understanding of the mechanism of action of electroconvulsive therapy in depression, and provide a basis for attempts to control seizures with adrenergic drugs.

ANNUAL REPORT

October 1, 1986 through September 30, 1987

Neuroepidemiology Branch

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
October 1, 1986 through September 30, 1987
Neuroepidemiology Branch
Division of Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke

Bruce S. Schoenberg, M.D., Dr.P.H., Chief

The Neuroepidemiology Branch is responsible for the development and implementation of epidemiologic and genetic programs to investigate the cause, prevention, and treatment of neurologic disorders in human populations. Emphasis has been placed on major neurologic diseases in which the diagnoses can be clinically verified to the satisfaction of skilled neurologists. The Branch is unique in being the only unit devoted exclusively to research in the epidemiology of diseases of the nervous system.

Neuroepidemiologic research studies require collaboration of many individuals. However, since there is a severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six videotapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared. In cooperation with the World Federation of Neurology Research Committee on Neuroepidemiology, a formal course was conducted in Beijing, the People's Republic of China in September, 1986. We are also providing opportunities for fellows to spend from six months to two years working with members of the Branch in order to learn the techniques of neuroepidemiology. During the past year we have had physicians from Thailand, India, Italy, the People's Republic of China, Canada, Tunisia, Japan, Ivory Coast, Nigeria, Venezuela, and Israel. Finally, current individual and institutional research training grant programs have been expanded to include neuroepidemiology. Institutional grants for training in neuroepidemiology have been awarded to Columbia University, New York, the University of California at Los Angeles, and Temple University, Philadelphia.

Epidemiologic studies have two basic requirements: uniformity and accuracy of data collection. This necessitates the use of a standardized, internationally accepted classification and coding system. The currently available scheme

published by the World Health Organization is seriously deficient with regard to neurologic disorders. The Branch is therefore collaborating with the World Health Organization Neurosciences Program, the World Federation of Neurology, and the American Academy of Neurology to revise this system of classification and improve its usefulness for neuroepidemiologic research. Two members of the Branch were selected to serve on the advisory committee to the World Health Organization to make recommendations for changes in this classification. A draft of the proposed changes has been prepared and has been circulated to neuroscientists from around the world for comments. However, since this new classification will not be available until 1992, the currently available scheme has been modified to make it more suitable for worldwide research in neuroepidemiology. This scheme will be published by the World Health Organization.

Another important problem for the neuroepidemiologist is the enormous cost of maintaining neurologic surveillance on a large number of patients. Therefore, we have attempted to utilize existing registries of neurologic diseases, such as in a study of presenile dementia based on the Israeli National Neurologic Disease Registry. In addition, we have assisted British investigators in organizing information routinely collected through the British National Health Service on all neurologic inpatients in a section of London with a population of 3-1/2 million inhabitants. The utility and accuracy of these data have been demonstrated in a study of the Guillain-Barre syndrome. A similar registry is being organized for the population of northeastern Italy, and another one in Hungary. We also collaborate with the Mayo Clinic in Rochester and utilize their record-linkage system to study neurologic diseases in the population of Rochester, MN.

Epilepsy is a major cause of morbidity and mortality on a world-wide basis. A considerable research effort is devoted by personnel of our Branch to studying this disease. It is our hypothesis that the different types of seizures have different risk factors, and that investigation of a single seizure type increases homogeneity of the case group and, therefore, the likelihood of identifying specific risk factors. Case-control studies were undertaken to determine risk factors for specific types of seizures, utilizing records from NIH, University of Virginia Hospitals and Walter Reed Army Medical Center.

In a case-control study of intractability of complex partial seizures, the following risk factors were identified: change in epilepsy pattern during the first two years after onset, history of head trauma, clusters of complex partial seizures, history of prolonged labor and multifocal paroxysms in the scalp EEG during the first two years after onset.

Studies of the relationship between ease of seizure control and prior duration of untreated epilepsy are being carried out in India and Venezuela. Untreated patients with epilepsy are being identified either through a door-to-door survey or from neurologic clinics. They are then placed on a standard local treatment regimen and their response to treatment monitored. All of these studies are using a uniform protocol.

Another neurologic disease of increasing importance in most of the developed countries, including the U.S., is Alzheimer's disease. This is another area of major research interest to our Branch personnel. Uniform diagnostic criteria are applied to all studies of Alzheimer's disease being conducted in our Branch. The clinical diagnosis is based on the criteria proposed by the NINCDS-Alzheimer's Disease and Related Disorders Association, Inc. work group. Where possible pathological verification of the diagnosis is obtained.

A careful review of the literature on dementia since 1907 has been completed. Special attention has been given to the cases of dementia originally described in Alzheimer's laboratory in Munich (West Germany). Using the United Nations population projections for the 20 year period 1980-2000, the possible effect of demographic trends on senile dementia prevalence in several "developed countries" (United Nations definition) has been studied. The estimated percent increase ranges from very low values in countries that will experience small demographic changes (France, Great Britain, and Sweden) to very high values in nations expected to undergo considerable changes in their elderly population (Italy 39.9%, U.S. 42.0%, and Japan 76.7%).

Four morbidity surveys of Alzheimer's disease are being or have been carried out. One is based on the records-linkage system of the Mayo Clinic which includes the entire population of Rochester, Minnesota. This study will enable us to estimate the incidence of Alzheimer's disease in a well-defined U.S. population. The second investigation utilized a two-stage survey consisting of a questionnaire and clinical examination. This survey has estimated the prevalence of severe dementia in Copiah County, Mississippi, which has equal proportions of black and white Americans. A third survey (in collaboration with the National Institute on Aging) is based on the on-going Honolulu Heart Project. This cohort of people have been followed prospectively for over 20 years and are now in the high risk age group for Alzheimer's disease. These people have been subject to repeated interviews and serum chemistry analyses. These prospectively collected data will be studied as risk factors for

Alzheimer's disease. In addition, some members of this cohort who are all of Japanese origin have come to autopsy. These cases will be intensively studied pathologically to identify the cause of dementia. The fourth survey of Alzheimer's disease has been conducted in Israel based on the nationwide registry of neurologic disease which exists for all Jewish residents of Israel. All cases diagnosed as having "dementia" of any etiology, between 1974 and 1978 were identified and the incidence rate of clinically diagnosed Alzheimer's disease determined based on the population age 60 and younger as well as for the different ethnic groups of this population.

In the study being conducted in Rochester, Minnesota, a neurologist using fixed diagnostic criteria, has reviewed records from all medical facilities serving the residents of Rochester, MN. This made it possible for the first time to determine the incidence of dementia coming to medical attention in a well-defined U.S. population. To confirm the reduced survival of demented patients reported on the basis of individuals hospitalized at specific medical centers, we have examined the survival of all demented individuals identified through this records-linkage study. Dealing with an entire population minimizes any possible selection bias that may be present for a series of patients seen at a particular medical institution. Data from this study are currently being analyzed in detail.

In the study conducted in Copiah County, it was found that for either sex, the prevalence ratios of all severe dementia of the Alzheimer's type were at least as large among blacks as among whites. For either race, the corresponding prevalence ratios were greater in females. For each race and sex, the corresponding prevalence ratios increased with advancing age. Finally, in the population studied, approximately 1% of individuals 40 years old or older had severe dementia. This figure increased to 7% for individuals 80 years old or older. The study in Honolulu is currently in progress. Clinical evidence of dementia has been obtained. Autopsies are being reviewed. The study conducted in Israel found that even among relatively younger individuals, rates increase with age and the risk is slightly greater among women. These findings are consistent with the epidemiologic patterns of Alzheimer's disease in older individuals. Also, a marked difference in the incidence rate of the disease has been found between Jews born in Europe-America (2.9/105) and those from Africa or Asia (1.4/105).

In addition, two case-control studies of Alzheimer's disease patients have been completed and two are in progress to determine risk factors for this disease. The first study uses cases and

controls selected from the Rochester, MN population. Past medical records have been utilized to obtain information concerning possible associations between Alzheimer's disease and either medical conditions or surgical procedures. This study has the advantage that recall bias cannot affect the results of the study since data are being abstracted from medical records. This study is in the advanced stages of data analysis. Three case-control studies of Alzheimer's disease utilizing interview data are being carried out in conjunction with a) the Alzheimer's Disease and Related Disorders Association, in Denver, Colorado, b) the Italian National Research Council, and c) The Burke Rehabilitation Center, White Plains, New York. The study in Denver and the one in Italy have been completed.

The case-control study in Denver has been completed. It was a study of late-onset Alzheimer's disease and was designed to test the hypothesis that risk factors may be different in early- and late-onset Alzheimer's disease. An interesting finding in this study is that in late-onset Alzheimer's disease, family history of dementia is not an important risk factor, thus suggesting that inheritance on a genetic basis may be more important in younger-onset cases than in older-onset cases.

The second case-control study conducted in Italy has also been completed. Two factors were found to be significantly associated with Alzheimer's disease - advanced age of the mother and a family history of dementia. Since the protocol utilized in Italy is similar to the one being used at the Burke Rehabilitation Center, White Plains, New York, international comparisons will be possible. This last case-control study is still in the process of data collection.

Yet another approach will utilize information obtained from clinical examination and combine it with autopsy data, thereby establishing a more definitive diagnosis of Alzheimer's disease. The objective of this study is to highlight the clinical characteristics which are most closely associated with pathologically proven Alzheimer's disease. This should help improve clinical diagnosis.

Epidemiologic studies of stroke have been ongoing by various Branch personnel for many years. The specific questions being currently addressed include: (1) what is the risk of stroke and transient ischemic attacks (TIA) in individuals with heart disease and/or hypertension as compared to the risk in individuals without these conditions; (2) whether the existence of pre-existing heart disease and/or hypertension affects the type of stroke and whether it affects survival following stroke; and (3) whether there is a particular time interval following the

onset of heart disease or hypertension during which an individual is at high risk for stroke. In addition, other studies address the issue of the effect of weather on stroke incidence, and a description of autopsy findings for patients dying with stroke in a defined community.

The first study involves a nonconcurrent prospective approach evaluating a cohort of 2,000 elderly individuals. The type of analysis follows the person-years strategy and utilizes life-table methods. This will be followed by an analysis using a time-dependent proportional hazards model to evaluate the individual contributions of hypertension and specific forms of cardiac disease to the risk of both transient ischemic attack and completed ischemic stroke. The investigations of weather variables and autopsy patterns are based on the records-linkage resource for residents of Rochester, MN.

When the case-control approach was applied to data available for the cohort of 2,000 individuals, different patterns of risk factors were demonstrated for TIA and completed ischemic stroke. While hypertension, diabetes mellitus, definite hypertensive heart disease, and valvular heart disease are important risk factors for completed stroke, these disorders have substantially less effect on the subsequent risk of TIA. When these data were analyzed in the format of a prospective study, it was possible to calculate the absolute risk of stroke as a function of the presence or absence of specific forms of cardiovascular disease. The following forms of cardiovascular disease yielded the highest ischemic stroke incidence rates (given in cases/1,000/year): myocardial infarction (15.5); congestive heart failure (20.5); and TIA (42.0). In considering risk factors for TIA, both angina/coronary insufficiency and congestive heart failure yielded the highest rates (10.4 and 10.9, respectively). Once etiologic precursors of stroke have been identified, medical intervention before the occurrence of long-lasting disability requires that there be an interval of time between the onset of the risk factor and the development of completed stroke. Analysis of data from this nonconcurrent prospective study revealed that those developing borderline hypertension, valvular heart disease, or ischemic heart disease remained stroke-free for the first 1-1/2 years after the first occurrence of each specific form of cardiovascular disease. This finding implies that there is an interval of time following the onset of these conditions when it may be possible to intervene medically to reduce the risk of stroke. Based on the time-dependent proportional hazards model, hypertension and transient ischemic attacks had the highest relative risks.

Other investigations in the area of stroke involve the

Careful analysis of unusual patterns of cerebrovascular disease (e.g., more than 20 TIA's/day). The study of weather variables and stroke revealed that temperature has no effect on stroke incidence.

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed mortality information on some neurologic diseases for the entire U.S. is not available. Analysis of mortality data can be particularly useful for some neurologic diseases because these may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated, or a contributory cause of death to get more complete information about the relationship between the disease and death. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. for many neurologic diseases. Diseases occurring together may provide important information in the search for etiology of diseases. Such detailed analysis of mortality data have been done for Alzheimer's disease and related diagnoses, motor neuron disease, hereditary ataxia, and Down's syndrome, for 1971-1978. The overall patterns which have emerged have been useful in evaluating trends over time and in formulating etiologic hypotheses.

All death certificates for the entire U.S. for the years 1971 and 1973 through 1978 were searched for the diagnosis of Huntington's Disease. Age-, race-, and sex-specific mortality rates for deaths due to and deaths with Huntington's disease were calculated. Time trends in the age-adjusted mortality rates between 1971 and 1978 were also calculated. To determine which conditions may be associated with reduced survival in patients with Huntington's Disease, all death certificates in the United States for 1978 on which Huntington's disease was mentioned have been studied. Each case was compared with two control deaths.

The age-adjusted mortality rates for Huntington's disease was higher for deaths with these conditions than due to them. Between 1971 and 1978, there was an increase in the age-adjusted mortality rates for Huntington's disease. Conditions associated with reduced survival in patients with Huntington's disease were: pulmonary infections, choking, nutritional deficiency, and chronic ulcer of skin.

Similar analyses are being conducted for stroke, motor neuron disease, Down's syndrome, and spina bifida.

Many different approaches have been utilized to study tumors of the nervous system. Descriptive epidemiologic techniques were applied to data obtained from tumor registries around the world. Wherever possible, cases were reviewed by the Branch's staff. New analytic techniques for studies of multiple primary cancers were devised. A case-control study is being done to estimate the association between putative risk factors and histologically confirmed grade III and IV astrocytomas in adults.

On the basis of the descriptive studies, two patterns of age-specific incidence emerged. Analyses of most population-based data worldwide revealed a small childhood peak, followed by a later peak between ages 50 and 80. Data for Rochester, MN, however, showed the childhood peak, followed by an increasing incidence rate with increasing age. Careful study of this discrepancy showed 1) that the greater percentage of cases first diagnosed at autopsy in Rochester accounted in large part for this difference, and 2) that a substantial number of brain tumors remain undiagnosed in the elderly during life. Studies are currently underway to evaluate the role of computerized tomography in the diagnosis of brain tumors and to explain the recent increase in the incidence of pituitary tumors among women of childbearing age. In addition, racial differentials in the frequency of certain intracranial tumors (meningiomas and pituitary adenomas) are being examined. Investigations of the relationship between intracranial neoplasms and extracranial tumors have been especially rewarding. An association was found between the occurrence of breast cancer and meningioma in women. This result raises interesting etiologic possibilities when considered with other evidence: 1) meningioma is the only common intracranial neoplasm with a higher incidence in females; 2) the abrupt clinical appearance or enlargement of this tumor during pregnancy has been described; and 3) the finding of estrogen receptor protein in meningioma has been reported. This has led to further work concerning estrogen receptors in other tumors such as malignant melanoma involving the nervous system.

There is continuing controversy regarding racial differences in the occurrence of major neurologic diseases. Thus, a study was conducted in Copiah County, Mississippi to specifically address this question. A strategy was developed which eliminated the requirement that persons must have entered the health care system for detection of disease. The basis of the investigation was a door-to-door survey which utilized a detailed questionnaire inquiring not only about diagnoses, but also about signs and symptoms suggestive of neurologic dysfunction. Over 99% of the households agreed to the interview. Those household members suspected of having one of the disorders of interest were then asked to have a neurologic examination conducted by a senior,

board-certified neurologist. This study showed that severe dementia of the Alzheimer's type is almost as common among black Americans as it is among white Americans. The prevalence ratio of Parkinson's disease is similar among blacks and whites residing in the same county in the U.S., whereas the prevalence of Parkinson's disease among blacks in West Africa is much lower. These findings suggest the importance of environmental factors in the etiology of Parkinson's disease.

Data from different parts of the world is often not directly comparable because of the use of different definitions of disease, different methodology to estimate disease frequency, and results are affected by availability of and access to experts in neurology. A protocol developed by the Branch in collaboration with the World Health Organization has eliminated these biases. The uniform protocol which is being used to estimate the prevalence of major neurologic disorders in many parts of the world, will enable international comparisons.

Pilot studies in Nigeria indicate that migraine is at least as common in rural black Africa as it is in urban populations of Western Europe. Furthermore, the prevalence of epilepsy is higher in rural Nigeria than reported for developed countries. In the People's Republic of China, the prevalence ratio of cerebrovascular disease is greater than anywhere else in the world where this disease has been studied.

The clinical neurogenetics component of the Branch involves three areas: 1) genetic-epidemiologic studies of movement disorders (e.g., the dystonias); 2) genetic-epidemiologic studies of multifactorial neurologic disorders (e.g., Parkinson's disease, Alzheimer's disease, and multiple sclerosis); and 3) genetic and biochemical studies of hereditary nervous system tumors.

Included among the disorders of movement such as the choreas, the dystonias, and tic syndromes are a number of discrete diseases which are due to a single gene mutation. Examples of mutations producing autosomal dominant traits are Huntington's chorea and one form of torsion dystonia. Examples of mutations leading to autosomal recessive traits are Lafora type myoclonic epilepsy, the newly described Baltic type myoclonus epilepsy, and the type of torsion dystonia probably responsible for most cases of dystonia in the Jewish population. Studies are being conducted to 1) uncover additional specific diseases within general movement disorder syndromes; 2) contribute to the understanding of their underlying biochemical basis; 3) determine the most effective treatment for each disorder; and 4) suggest guidelines for counseling individual family members.

Initially, families with members exhibiting a particular syndrome undergo detailed clinical evaluation. Extensive genealogical data are then analyzed in conjunction with clinical observations and relevant laboratory studies. A nosologic classification is prepared. Promising biochemical leads, particularly involving neurotransmitter metabolism, are explored in collaboration with established investigators. Simultaneously, existing treatment programs are evaluated, and where indicated, there are therapeutic trials of new agents. Based on low CSF biopterin in a form of familial dystonia, biopterin has been administered intravenously to 10 patients resulting in transient improvement in several.

Together with colleagues at Johns Hopkins Hospital we have reported the first example of Munchausen Syndrome presenting as torsion dystonia. The patient underwent brain surgery, tracheostomy, and feeding gastrostomy before she told us of the factitious basis for her dystonia-like symptoms.

Our goal is to determine the relative genetic and environmental contribution to multifactorial diseases. Focus is on likely biochemical or immunogenetic abnormalities suggesting a specific genetic role coupled with the search for environmental risk factors. Approaches and techniques used are from multiple disciplines including clinical, population, and biochemical genetics coupled with descriptive and analytical epidemiology. Selected populations including families with multiple members or twin pairs affected with the disease are studied in depth. Unaffected family members, unaffected twins, and spouses serve as controls.

What may be a new adult onset leukodystrophy but associated with major autonomic system disturbance and diagnosable by CT and MRI scan has been mistaken for MS in at least 20 members of one kindred. Derangement of the autonomic nervous system is often seen early in the course and when recognized, serves to distinguish this single gene disorder clinically from multiple sclerosis of the chronic progressive type. Extensive studies were performed on 9 at-risk individuals, 8 offspring, ages 23 to 32, and one 58-year-old sibling. Evoked potentials (BAEP's, VEP's, and SEP's) and physiologic autonomic testing were normal in all nine. A 32-year-old asymptomatic male had subtle pyramidal findings on examination with CT/MRI changes in the frontoparietal and cerebellar white matter, consistent with an early stage of the disorder. Another 30-year-old male had a site of increased signal intensity in the parietal-occipital area on MRI. A long history of constipation and urinary frequency was elicited in a 25-year-old female with normal neuroradiologic evaluation.

Sixty-two twin pairs with Parkinson's disease have now been evaluated. Among 43 monozygotic pairs, there is only one definite concordant pair and there are no definitely concordant pairs among 19 dizygotic pairs, suggesting that the genetic contribution in Parkinson's disease is not the decisive factor in twins. There appears to be a lifelong personality difference between affected and unaffected twins, the affected twin being less outgoing. Smoking is also less frequent in the affected twins, but this may reflect personality difference. A simple, novel, etiologic theory involving initial neuron number has been suggested based on these findings.

The initial phase of a multidisciplinary study of Alzheimer's disease occurring in 22 twin pairs has been completed. Of 17 monozygotic pairs, 7 were concordant. The average duration of disease in the affected member of the 10 discordant pairs was 8 years with a range of 5 to 10 years. Of 5 dizygotic twin pairs, 2 were concordant. The duration of disease in the 3 discordant pairs was 10 years, with a range of 6 to 15 years. Autopsy verification of diagnosis was made in 4 affected twins.

There are at least ten genetically determined syndromes which include as one of their chief manifestations tumors of the nervous system. Neurofibromatosis and tuberous sclerosis are among the more common examples. The objective of another project is to 1) document additional hereditary traits which can cause such neoplasms; 2) add information to the clinical description and natural history of such traits; 3) suggest effective means of early diagnosis; 4) evaluate various modes of treatment; and 5) develop methods of preclinical detection and screening. In families with multiple individuals affected with the same rare tumor of the nervous system, members undergo clinical, genetic, and psychological evaluation. Appropriate physiologic and biochemical studies are carried out in collaboration with laboratory investigators.

We have documented the existence of a distinct form of neurofibromatosis, the hallmark of which is bilateral acoustic neuroma, in addition to the type described by von Recklinghausen. Currently, we are evaluating the usefulness of standard audiologic studies, acoustic reflex decay, brain stem auditory-evoked potential, CT scan and nuclear magnetic resonance in screening at-risk individuals, establishing a diagnosis, and managing those with known bilateral acoustic neuromas. Preliminary results suggest brain stem auditory-evoked potential and acoustic reflex decay are most useful for screening and for monitoring those with small, slowly growing tumors. An additional screening approach we suggest consists of bio-ophthalmoscopic study of the lens. Six of 23 patients

exhibited posterior subcapsular opacities which in several instances antedated audiologic or vestibular changes.

Mental retardation, attention deficit disorder, and learning disabilities are considered to be frequent features of von Recklinghausen's neurofibromatosis. However, there are few reports of formal neuropsychological testing of these individuals and none involving rigorous selection of controls. A pilot study evaluating neurological and cognitive function in neurofibromatosis has been performed. Following local announcements, thirteen sibling pairs between the ages of 6 and 26, one affected with neurofibromatosis and one unaffected, were selected. By design, none of the affected individuals had evidence of central nervous system pathology.

The neurofibromatosis cases scored significantly higher in number of subtle neurological abnormalities ($p < 0.01$) and significantly lower in Full Scale IQ ($p < 0.01$) than their unaffected siblings. Their IQ scores were not clustered at the lower end of the scale, but rather, there was a consistent shift downward in their IQ distribution when compared with their siblings. In addition, a deficit in visual-spatial orientation was present in cases ($p < 0.025$). No significant excess of low cognitive function, attention deficit disorder, or learning disabilities were found.

In the field of pediatric neuroepidemiology a study of mental retardation is being conducted in Rochester, Minnesota, using their records-linkage system. The objectives of this study are to determine the incidence of mental retardation, temporal trends in incidence and risk factors for mental retardation in this population.

Though emphasis is placed on research on the major diseases of the nervous system, other diseases which may be less frequent or less debilitating but important in terms of pathogenesis or clues to disease etiology, are also under investigation in our Branch.

In collaboration with other governmental agencies (Centers for Disease Control), other governments (Colombia, Republic of Seychelles), international organizations (World Health Organization), and universities (Mayo Medical School) methods have been developed to investigate less common neurologic disorders. Several such studies have been completed. Current projects include investigation of space/time clusters of neurologic disease (with the Centers for Disease Control), clusters of multiple sclerosis (with the Multiple Sclerosis Society), a study of myasthenia gravis and multiple sclerosis in

the same patient (with the Mayo Clinic), an investigation of neurologic disorders during pregnancy and the postpartum period (with the Mayo Clinic), an investigation of pseudotumor cerebri diagnosed at the University of Illinois, Chicago, a prevalence and case-control study of normal pressure hydrocephalus (with VA Medical Center, Shreveport, Louisiana), a prevalence and case-control study of progressive supranuclear palsy (with University of Medicine and Dentistry of New Jersey), descriptive and analytic studies of spastic paraparesis in different parts of the world, and a study of risk factors for cerebral malaria.

Headaches are an important cause of disability in the general population. However, because of problems with definitions and methodology of survey, reliable population based data on the prevalence of headaches is not available. Surveys have been conducted in Mexico and the People's Republic of China to estimate the prevalence of headaches. These studies have utilized a uniform protocol, thus international comparisons will be possible. The study of headaches in China showed that the prevalence of "incapacitating headaches" was about twice as common in women compared to men and also about twice as common in the urban areas compared to rural areas.

The study of pseudotumor cerebri showed that low protein in the cerebrospinal fluid (CSF) often reported in these patients is related to high CSF pressure. This study documented an inverse linear relationship between CSF protein content and CSF opening pressure at the time of lumbar puncture. Further studies are being conducted to elucidate the natural history of pseudotumor cerebri using MRI scans.

A special study of spastic paraparesis in Tumaco, Colombia (along the southern Pacific coast of Colombia) was carried out. The clinical and epidemiologic features of the disease were characterized and special case-control studies to address etiologic factors were initiated. In a special study of tropical spastic paraparesis in the Seychelles Islands, we discovered a strong association between this disease and antibodies to human T-lymphotropic virus-Type I (HTLV-I).

In collaboration with the U.S. Army, a retrospective cohort approach will be used to study the problem of cerebral malaria. A cohort of individuals who served in the military in Viet Nam from 1968-1970, will be identified and those who can be reached will be examined by questionnaire, physical examination, and psychometric testing.

Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These

yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been given to: cerebrovascular disease; otitis media; inherited ataxias; Huntington's disease; febrile seizures; Tourette's syndrome; peripheral neuropathy; neurologic disease in the elderly; controlled therapeutic trials of motor neuron disease; epilepsy; descriptive, analytic, and experimental methods in neuroepidemiology; statistical methods for calculating confidence intervals, procedures for neuroepidemiologic investigations in developing countries; and epidemiologic studies of Alzheimer's disease; myasthenia gravis; and cerebral malaria.

The efficacy and side effects of phenobarbital in young children with febrile seizures, randomized to phenobarbital or placebo is being studied. This is the last year of data collection. A number of endpoints will be assessed: IQ, seizure recurrence, sleep pattern, temperament scale, and a number of behavioral and cognitive measures.

Studies of dental and dermatoglyphic markers of maldevelopment have been initiated. This is a study of enamel defects and characteristics of dermatoglyphics and palmar creases in children with cerebral palsy or mental retardation, in cooperation with the National Institute of Dental Research and the Institute of Aging. This is an attempt to recognize the occurrence and timing of events adverse to neurologic development. A local study has been initiated to identify parameters for a case-control study to be performed in a defined population in California.

A pilot study for the exploration of the utility of dental and dermal markers was completed. Dental pits were observed in 3 of 34 severely retarded children. Pits of this description have been said to be pathognomonic of tuberous sclerosis, though that was not the clinical diagnosis in any of these children. A study has been initiated to examine a group of self-identified or family-identified patients with tuberous sclerosis for enamel pits. The purpose of this study will be to explore with what frequency dental enamel findings can add diagnostic information in tuberous sclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01924-17 NEB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Genetic, Pathophysiologic Study of Hereditary Movement Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Roswell Eldridge, M.D. Ronald J. Polinsky, M.D.	Medical Geneticist Neurologist	NEB, DIR, NINCDS MNB, DIR, NINCDS
COOPERATING UNITS (if any) MNB, DIR, NINCDS; HE, NHLBI; LCS, DCBR, NIMH		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.3	PROFESSIONAL: 0.3	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In this project, we seek to 1) clarify and expand the nosology of the hereditary movement disorders; 2) contribute to the understanding of the underlying biochemical basis; 3) determine the most effective treatment for each disorder; and 4) suggest guidelines for counseling individuals at risk. General syndromes under study include the dystonias, tic disorders, blepharospasm, and myoclonus. Approaches include standard epidemiologic and clinical genetic studies together with collaborative efforts in evaluating the role of neurotransmitters such as dopamine, their precursors, and metabolites, and their necessary cofactors. Collaborative studies are underway with personnel in LCS, DCBR, NIMH and HE, NHLBI to explain our earlier observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and biopterin in CSF in a genetic subset of dystonia patients. Members of selected families are admitted to the Clinical Center, NIH, for trial of several new pharmacological agents. Biopterin administered intravenously has led to acute benefit in one form of generalized dystonia. Publications for FY 1987 are listed with the NEB report.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01927-17 NEB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Roswell Eldridge, M.D. Martha Denkla, M.D. Ellen Bien, Ph.D. Anita Pikus Donald Wright, M.D.	Medical Geneticist Child Neurologist Clinical Psychologist Audiologist Medical Officer	NEB, DIR, NINCDS NEB, DNB, NINCDS NEB, NINCDS OP, CC, NIH SN, DIR, NINCDS
COOPERATING UNITS (if any) OP, CC: SN, DIR, NINCDS: Division of Medical Genetics, Dept. of Pediatrics, Children's Hospital National Medical Center; Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In this project we seek to define and classify hereditary tumors of the nervous system such as occur in neurofibromatosis; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment; and to develop methods for preclinical detection and screening. Our studies have led to the recognition of at least two distinct genetic forms of neurofibromatosis: 1) the classical form as described by von Recklinghausen, and 2) a form in which bilateral acoustic neuromas are the hallmark. Efforts in the latter have been directed at improving and simplifying screening of high-risk individuals, confirming diagnosis, and establishing criteria for intervention. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are useful means for early documentation and monitoring of acoustic neuromas. We have recently found that presenile lens opacities or cataracts occur in about 50% of such patients. In our first major study involving neurofibromatosis of the von Recklinghausen type, a multi-disciplinary project is nearing completion to evaluate neurologic and cognitive status in these patients compared to their unaffected sibs. We plan to initiate gene-linkage studies in neurofibromatosis, so successful in Huntington disease. Publication is listed with NEB report.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02167-13 NEB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Epidemiology Studies in MS and Other Multifactorial Neurologic Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Rowell Eldridge, M.D. Henry F. McFarland, M.D. Ronald J. Polinsky, M.D. Walter A. Rocca	Medical Geneticist Assistant Chief Neurologist Visiting Associate	NEB, DIR, NINCDS NI, DIR, NINCDS MNB, DIR, NINCDS NEB, NINCDS
COOPERATING UNITS (If any) BFSB, NEB, NI, MNB, NINCDS; M, CN, NIMH; Department of Neurology, Monmouth Medical Center, Monmouth, NJ		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.4	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In this project we are coupling genetic and environmental studies in selected families and twin pairs with disorders such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease, in an effort to distinguish specific contributing factors. A multi-disciplinary twin study of Parkinson's disease has led to formulation of an etiologic theory we term the "initial neuron number" hypothesis. Since neurons in the substantia nigra are not known to regenerate but rather appear to die off at a constant rate during adulthood, starting life with a reduced number of these critical neurons may be one predisposing factor to eventual development of the disorder. A study similar in design involving twins with dementia of the Alzheimer's types also indicates environmental factors must be involved in some forms of the disorder. An autosomal dominant, hereditary leukoencephalopathy simulating MS with onset at about age 35 is under study in a kindred with over 20 affected. Derangement of the autonomic nervous system is often seen early in the course and when recognized clinically, serves to distinguish this single gene disorder from multiple sclerosis. Computerized tomographic scan changes of the brain are characteristic, even in early cases. Publications for FY 1987 are listed with the NEB report. 17 -- NEB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02240-11 NEB												
PERIOD COVERED October 1, 1986 through September 30, 1987														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Epidemiology of Dementia														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Bruce S. Schoenberg, M.D., Dr.P.H.</td> <td style="width: 30%;">Chief,</td> <td style="width: 30%;">NEB, DIR, NINCDS</td> </tr> <tr> <td>Vijay Chandra, M.D., Ph.D.</td> <td>Medical Officer,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Walter Rocca, M.D., M.P.H.</td> <td>Visiting Scientist,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Therese Treves, M.D.</td> <td>Visiting Scientist,</td> <td>NEB, DIR, NINCDS</td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS	Vijay Chandra, M.D., Ph.D.	Medical Officer,	NEB, DIR, NINCDS	Walter Rocca, M.D., M.P.H.	Visiting Scientist,	NEB, DIR, NINCDS	Therese Treves, M.D.	Visiting Scientist,	NEB, DIR, NINCDS
Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS												
Vijay Chandra, M.D., Ph.D.	Medical Officer,	NEB, DIR, NINCDS												
Walter Rocca, M.D., M.P.H.	Visiting Scientist,	NEB, DIR, NINCDS												
Therese Treves, M.D.	Visiting Scientist,	NEB, DIR, NINCDS												
COOPERATING UNITS (if any) Epidemiology, Demography, and Biometry, NIA; W. Massey, M.D., Duke Univ.; E. Kokmen, M.D., and J.P. Whisnant, M.D., Mayo Clinic; B. Jordan, Columbia University; NY														
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research														
SECTION														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS 1.3	PROFESSIONAL: 1.3	OTHER:												
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☒ (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Population-based estimates of the incidence, prevalence, morbidity and mortality of Alzheimer's disease for the U.S. have not been available. A study has recently been completed for the entire population of Rochester, Minnesota where the incidence of Alzheimer's disease has been estimated. This is the first study of its kind in the U.S. Another study to estimate the prevalence of "severe dementia" in a population with equal proportions of black and white Americans has also been completed and published. National mortality data for the entire U.S. for the period 1971, 1973-1978 for Alzheimer's disease and related diagnoses has also been analyzed for the first time and recently published. Analytic studies to determine risk factors for Alzheimer's disease are being conducted. A case-control study of late-onset Alzheimer's disease has been completed in Denver, Colorado. Another large case-control study has been completed in Rochester, MN. Another case-control study of Alzheimer's disease is in progress in White Plains, New York. A multi-center case-control study has also been completed in Italy. Since this study utilized a similar protocol to that used in the U.S., international comparisons are possible. A historical review of the distinction between "Alzheimer's disease" and "senile dementia" has been done. Projections for the prevalence of senile dementia in the year 2000 for several developed countries have been developed. An attempt has been made to evaluate and improve instruments used in case-control studies of dementia. Publications for FY 1987 are listed with the NEB report.														

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02241-11 NEB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Epidemiology of Cerebrovascular Disease in Adults

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bruce S. Schoenberg, M.D., Dr.P.H.

Chief,

NEB, DIR, NINCDS

Patricia Davis (Lew), M.D.

Guest Researcher,

NEB, DIR, NINCDS

James Dambrosia, Ph.D.

Chief,

BFSB, DIR, NINCDS

COOPERATING UNITS (if any)

J.P. Whisnant, M.D., Mayo Clinic; D.G. Schoenberg, M.S., Bethesda, Maryland;
A. Lilienfeld, M.D., Johns Hopkins University

LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This investigation is aimed 1) at evaluating the effect of heart disease and hypertension as potentially treatable precursors of completed stroke and transient ischemic attacks; 2) at documenting unusual patterns of cerebrovascular disease; 3) at determining the autopsy patterns for patients dying with cerebrovascular disease in defined community; and 4) at examining if weather parameters have any effect on stroke incidence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02243-11 NEB																		
PERIOD COVERED October 1, 1986 through September 30, 1987																				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pediatric Neuroepidemiology																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Bruce S. Schoenberg, M.D., Dr.P.H.</td> <td style="width: 33%;">Chief,</td> <td style="width: 33%;">NEB, DIR, NINCDS</td> </tr> <tr> <td>Vijay Chandra, M.D., Ph.D.</td> <td>Medical Officer,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Lawrence Lavine, DO, MPH, DTM & H</td> <td>Medical Officer,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Zhao Fu, M.D.</td> <td>Guest Researcher,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Sherry Emoto, Ph.D.</td> <td>Staff Fellow,</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Karin B. Nelson, M.D.</td> <td>Medical Officer,</td> <td>NEB, DIR, NINCDS</td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS	Vijay Chandra, M.D., Ph.D.	Medical Officer,	NEB, DIR, NINCDS	Lawrence Lavine, DO, MPH, DTM & H	Medical Officer,	NEB, DIR, NINCDS	Zhao Fu, M.D.	Guest Researcher,	NEB, DIR, NINCDS	Sherry Emoto, Ph.D.	Staff Fellow,	BFSB, DIR, NINCDS	Karin B. Nelson, M.D.	Medical Officer,	NEB, DIR, NINCDS
Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS																		
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Sherry Emoto, Ph.D.	Staff Fellow,	BFSB, DIR, NINCDS																		
Karin B. Nelson, M.D.	Medical Officer,	NEB, DIR, NINCDS																		
COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J.F. Mellinger, M.D., M.R. Gomez, M.D., L.T. Kurland, M.D., Dr.P.H. and R.V. Groover, M.D., Dept. of Neurology, Mayo Clinic; L.L. Salkowicz; P. Gunderson, Ph.D., Minnesota Dept. of Health;*																				
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research																				
SECTION																				
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																				
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:																		
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A study of mental retardation is being conducted in Rochester, Minnesota, using their records-linkage system. The objectives of this study are to determine the incidence of mental retardation, temporal trends in incidence and risk factors for mental retardation in this population. Analysis of data for febrile seizures is being conducted based on a large prevalence survey from the People's Republic of China. Publications for FY 1987 are listed with the NEB report. ----- *Continued <table style="width: 100%; border: none; margin-top: 20px;"> <tr> <td style="width: 33%;">M. Cruz, M.D.</td> <td style="width: 33%;">Mayo Clinic</td> <td style="width: 33%;">Rochester, MN</td> </tr> <tr> <td>F. Baker, M.D.</td> <td>USPHS Epidemiology Training Program</td> <td>Bethesda, MD</td> </tr> </table>			M. Cruz, M.D.	Mayo Clinic	Rochester, MN	F. Baker, M.D.	USPHS Epidemiology Training Program	Bethesda, MD												
M. Cruz, M.D.	Mayo Clinic	Rochester, MN																		
F. Baker, M.D.	USPHS Epidemiology Training Program	Bethesda, MD																		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02297-11 NEB		
PERIOD COVERED October 1, 1986 through September 30, 1987				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mortality from Neurologic Disorders: National and International Comparisons				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> Bruce S. Schoenberg, M.D., Dr.P.H. Chief, Vijay Chandra, M.D., Ph.D. Medical Officer, Lawrence Lavine, M.D., DO, MPH, DTM&H Medical Officer, Maurizio Leone, M.D. Guest Researcher, Hisaharu Suzuki, M.D. Guest Researcher, Therese Treves, M.D. Guest Researcher, Satish Chandra, Parthasathy, M.D. Guest Researcher, Adesola Ogunniyi, M.D. Guest Researcher, Douglas Lanska, M.D. Special Volunteer, </td> <td style="width: 50%; vertical-align: top; text-align: right;"> NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS </td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H. Chief, Vijay Chandra, M.D., Ph.D. Medical Officer, Lawrence Lavine, M.D., DO, MPH, DTM&H Medical Officer, Maurizio Leone, M.D. Guest Researcher, Hisaharu Suzuki, M.D. Guest Researcher, Therese Treves, M.D. Guest Researcher, Satish Chandra, Parthasathy, M.D. Guest Researcher, Adesola Ogunniyi, M.D. Guest Researcher, Douglas Lanska, M.D. Special Volunteer,	NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS
Bruce S. Schoenberg, M.D., Dr.P.H. Chief, Vijay Chandra, M.D., Ph.D. Medical Officer, Lawrence Lavine, M.D., DO, MPH, DTM&H Medical Officer, Maurizio Leone, M.D. Guest Researcher, Hisaharu Suzuki, M.D. Guest Researcher, Therese Treves, M.D. Guest Researcher, Satish Chandra, Parthasathy, M.D. Guest Researcher, Adesola Ogunniyi, M.D. Guest Researcher, Douglas Lanska, M.D. Special Volunteer,	NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS			
COOPERATING UNITS (if any) W. Massey, M.D., Duke University; D.G. Schoenberg, M.S., Bethesda, Maryland				
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research				
SECTION				
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892				
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.7	OTHER:		
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed mortality information on some neurologic diseases for the entire U.S. is not available. Analysis of mortality data can be particularly useful for some neurologic diseases because these may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated, or contributory cause of death to get more complete information about the relationship between the disease and death. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. for many neurologic diseases. Diseases occurring together may provide important information in the search for etiology of diseases. Such detailed analysis of mortality data have been done for Alzheimer's disease and related diagnosis, motor neuron disease, Down's syndrome, spina bifida, hereditary ataxias, Huntington's disease, epilepsy, strokes in patients without hypertension, multiple sclerosis, and Parkinson's disease, for 1971, 1973-1978. The overall patterns which have emerged have been useful in evaluating trends over time and in formulating etiologic hypotheses.				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02299-11 NEB			
PERIOD COVERED October 1, 1986 through September 30, 1987					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Reviews of Epidemiology Aspects of Neurologic Disease					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%; vertical-align: top;"> Bruce S. Schoenberg, M.D., Dr.P.H. Lawrence Lavine, DO, MPH, DTM & H Walter A. Rocca, M.D., M.P.H. Patricia Davis (Lew), M.D. Therese Treves, M.D. </td> <td style="width: 40%; vertical-align: top;"> Chief, Medical Officer, Visiting Associate, Guest Researcher, Visiting Scientist, </td> <td style="width: 20%; vertical-align: top;"> NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS </td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H. Lawrence Lavine, DO, MPH, DTM & H Walter A. Rocca, M.D., M.P.H. Patricia Davis (Lew), M.D. Therese Treves, M.D.	Chief, Medical Officer, Visiting Associate, Guest Researcher, Visiting Scientist,	NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS
Bruce S. Schoenberg, M.D., Dr.P.H. Lawrence Lavine, DO, MPH, DTM & H Walter A. Rocca, M.D., M.P.H. Patricia Davis (Lew), M.D. Therese Treves, M.D.	Chief, Medical Officer, Visiting Associate, Guest Researcher, Visiting Scientist,	NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS			
COOPERATING UNITS (if any) D. Schoenberg, M.S., Bethesda, Maryland; N. Bharucha, M.D., Bombay, India; L. Amaducci, M.D., Firenze, Italy					
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research					
SECTION					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892					
TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.7	OTHER:			
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been given to: cerebrovascular disease; otitis media; inherited ataxias; Huntington's disease; febrile seizures; Tourette's syndrome; peripheral neuropathy; neurologic disease in the elderly; controlled therapeutic trials of motor neuron disease; epilepsy; descriptive, analytic, and experimental methods in neuroepidemiology; statistical methods for calculating confidence intervals; procedures for neuroepidemiologic investigations in developing countries; and epidemiologic studies of Alzheimer's disease; myasthenia gravis; and cerebral malaria. Publications for FY 1987 are listed with NEB report.					

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02300-11 NEB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Course and Medical Care for Neurologic Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bruce S. Schoenberg, M.D., Dr.P.H. Chief,

NEB, DIR, NINCDS

COOPERATING UNITS (if any)

J.P. Whisnant, M.D., Department of Neurology, Mayo Clinic, Rochester, MN

LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study uses a review and abstraction of data from records for a selected group of neurological disorders. It obtains the items of data necessary to determine onset of the disorder, duration, date and cause of death, or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve, and morbidity and severity estimates. It will also include analysis of type and duration of medical care received by patients with neurologic disorders derived from a well-defined population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02301-11 NEB										
PERIOD COVERED October 1, 1986 through September 30, 1987												
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Collaborative Studies of Less Common or Less Debilitating Neurologic Disorders												
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)												
Bruce S. Schoenberg, M.D., Dr. P.H. Lawrence Lavine, DO, MPH, DTM & H Patricia Davis, M.D. Vijay Chandra, M.D., Ph.D.	Chief, Medical Officer, Visiting Scientist, Medical Officer,	NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS NEB, DIR, NINCDS										
COOPERATING UNITS (if any) M. Zack, M.D., Atlanta, Georgia; Neurosciences Program, WHO, Geneva, Switzerland; D. Duane, M.D., B. Sandok, M.D., Mayo Clinic; G. Roman, Lobbuck, Texas; P.S. Spencer, Albert Einstein College of Medicine, New York; R. Duviosin, M.D., Univ. of Medicine & Dentistry of New Jersey, New Brunswick, NJ*												
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research												
SECTION												
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892												
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:										
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews												
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)												
A number of collaborative efforts involve investigation of the characteristics of unusual or less debilitating (e.g., headache) neurologic disease phenomena. Unusual associations or space/time clusters of neurologic disorders may provide leads to etiology or therapy. These may be tested through more formal approaches. A study of over 100 cases of pseudotumor cerebri has been completed. This study documented an inverse linear relationship between cerebrospinal fluid (CSF) opening pressure and CSF protein content. Further analysis of these data are in progress. Descriptive and analytic studies of spastic paraparesis are being conducted in Columbia, India, the Seychelles Islands, and the West Indies. A retrospective cohort approach is being used to investigate the sequelae of cerebral malaria due to infection with Plasmodium Falciparum. Surveys for Parkinson's disease are being conducted in former Far East prisoners of war. Publication for FY 1987 is listed with NEB report. ----- *Continued <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">L. Golbe, M.D.</td> <td style="width: 60%;">Univ. of Medicine &</td> </tr> <tr> <td></td> <td>Dentistry of New Jersey New Brunswick, NJ</td> </tr> <tr> <td>P. Kark, M.D.</td> <td>V.A. Medical Center Shreveport, LA</td> </tr> <tr> <td>C. Tanner, M.D.</td> <td>Rush Presbyterian Med.Ctr.Chicago, IL</td> </tr> <tr> <td>C. Goetz, M.D.</td> <td>" " " "</td> </tr> </table>			L. Golbe, M.D.	Univ. of Medicine &		Dentistry of New Jersey New Brunswick, NJ	P. Kark, M.D.	V.A. Medical Center Shreveport, LA	C. Tanner, M.D.	Rush Presbyterian Med.Ctr.Chicago, IL	C. Goetz, M.D.	" " " "
L. Golbe, M.D.	Univ. of Medicine &											
	Dentistry of New Jersey New Brunswick, NJ											
P. Kark, M.D.	V.A. Medical Center Shreveport, LA											
C. Tanner, M.D.	Rush Presbyterian Med.Ctr.Chicago, IL											
C. Goetz, M.D.	" " " "											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02305-11 NEB						
PERIOD COVERED October 1, 1986 through September 30, 1987								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Epidemiology of Intracranial Neoplasms								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)								
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Bruce S. Schoenberg, M.D., Dr.P.H.</td> <td style="width: 30%;">Chief,</td> <td style="width: 30%;">NEB, DIR, NINCDS</td> </tr> <tr> <td>Patricia Davis</td> <td>Guest Researcher,</td> <td>NEB, DIR, NINCDS</td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS	Patricia Davis	Guest Researcher,	NEB, DIR, NINCDS
Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS						
Patricia Davis	Guest Researcher,	NEB, DIR, NINCDS						
COOPERATING UNITS (if any) B.W. Christine, M.D., M.P.H., Connecticut State Department of Health; J.P. Whisnant, M.D., and R.J. Campbell, M.D., Mayo Clinic; L. Mahalak, M.D., Jackson, MS; A. Heck, M.D., Univ., of TN; R. Simon, M.D., Berkley, CA; B. Jordan, B.A., Harvard Medical School								
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research								
SECTION								
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892								
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER:						
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews								
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Branch has conducted extensive investigations on the descriptive epidemiology of primary intracranial neoplasms using data from population-based registries worldwide. Analytic studies were carried out to investigate the relationship between intracranial neoplasms and tumors occurring at other sites. These studies included careful review of tumor nomenclature, disease definitions, and survey strategies. A case-control study of glioblastoma multiforme is now being conducted.								

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02307-11 NEB
PERIOD COVERED <u>October 1, 1986 through September 30, 1987</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Educational Resources in Neurological Epidemiology</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bruce S. Schoenberg, M.D., Dr.P.H. Vijay Chandra, M.D., Ph.D. Chief, Medical Officer, NEB, DIR, NINCDS NEB, DIR, NINCDS		
COOPERATING UNITS (if any) <u>D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland</u>		
LAB/BRANCH <u>Neuroepidemiology Branch, Division of Intramural Research</u>		
SECTION 		
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <u>0.1</u>	PROFESSIONAL: <u>0.1</u>	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) Because there is severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six video tapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared. In cooperation with the World Health Organization and the World Federation of Neurology Research Group on Neuroepidemiology, a formal course was conducted in Hamburg, West Germany. Another course was held in Beijing, The People's Republic of China. A set of video tapes have been produced for training interviewers in the methodology of interviewing for case-control studies. This has been done in both Italian and in English.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02370-09 NEB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Racial and Geographic Differences in Occurrence of Neurologic Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bruce S. Schoenberg, M.D., Ph.D., Chief, NEB, DIR, NINCDS Vijay Chandra, M.D., Ph.D., Medical Officer, NEB, DIR, NINCDS Lawrence Lavine, D.O., M.P.H., D.T.M. & H., Medical Officer, NEB, DIR, NINCDS P. Satish Chandra, M.D., Guest Researcher, NEB, DIR, NINCDS Beatrice Guerra, M.D., Guest Researcher, NEB, DIR, NINCDS Beugre Kouassi, M.D., Guest Researcher, NEB, DIR, NINCDS Zhao Fu, M.D., Guest Researcher, NEB, DIR, NINCDS Neila Romdhane, M.D., Guest Researcher, NEB, DIR, NINCDS Adesola Oggunyi, M.D., Guest Researcher, NEB, DIR, NINCDS		
COOPERATING UNITS (if any) OBFS, OD, NINCDS: A. Haerer, M.D., Univ. of Mississippi; U.S. Bureau of the Census; C.L. Bolis, M.D., (WHO); B.O. Osuntokun, M.D. (Nigeria); F. Garcia-Pedroza, M.D., (Mexico); Wang Chung-cheng, M.D. (People's Republic of China); N. Bharucha, M.D. (India); M.C. Gutierrez del Olmo, M.D.;**		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.2	PROFESSIONAL: 4.2	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☒ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this study is to accurately document possible racial differentials in the prevalence of major neurologic disorders by surveying an entire county, with a biracial population of approximately 25,000. The disorders investigated include cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, and cerebrovascular disease. In addition, research protocols for neuroepidemiologic studies in developing countries have been prepared for Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, Spain, Venezuela, and India. Pilot investigations have been successfully carried out in Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, and India. *[Former title: Racial Differentials in the Prevalence of Major Neurologic Disorders and Surveys in Developing Countries]. Publications for FY 1987 are listed with NEB report. ----- **Continued A. Portera-Sanchez, M.D., J. Cabrera, M.D. P. Ponce, M.D. M. Cruz, M.D. (Spain) (Peru) (Venezuela) (Ecuador)		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02423-08 NEB															
PERIOD COVERED October 1, 1986 through September 30, 1987																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Data Resources for Neuroepidemiology																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)																	
Bruce S. Schoenberg, M.D., Dr.P.H. Chief, Lawrence Lavine, DO, MPH, DTM & H Medical Officer,		NEB, DIR, NINCDS NEB, DIR, NINCDS															
COOPERATING UNITS (if any) F. Clifford Rose, M.B., FRCP; B. Benjamin, Ph.D., S. Haberman, M.A., F.I.A., and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England; W. Sibley, M.D., Univ. of Arizona, Tucson, AZ; E. Kahahah, M.D., Neurology Unit, Ashkelon, Israel;*																	
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research																	
SECTION																	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:															
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) To develop 1) a registry of hospitalized patients with neurologic disease in a well-defined population of 3.5 million people, 2) resources for case-control studies of neurologic diseases using uniform methods of data collection, 3) to develop a registry of neurologic disease in the well-defined population of the United States military, and 4) a registry of selected neurologic diseases in a well-defined population of 0.5 million people in a county in Hungary.																	

*Continued <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Y. Leibowitz</td> <td style="width: 40%;">Neuroepidemiology Unit</td> <td style="width: 20%;">Jerusalem, Israel</td> </tr> <tr> <td>G. Palffy, M.D.,</td> <td>Neurology, Univ. of Pecs</td> <td>Pecs, Hungary</td> </tr> <tr> <td>C. Gunderson, Col., M.C.(Army)</td> <td>Dept. of Neurology</td> <td>Walter Reed Army Med. Center</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>J. Hallenbeck, CAPT., M.C.,(Navy)</td> <td>Dept. of Neurology</td> <td>Bethesda Naval Hosp.</td> </tr> </table>			Y. Leibowitz	Neuroepidemiology Unit	Jerusalem, Israel	G. Palffy, M.D.,	Neurology, Univ. of Pecs	Pecs, Hungary	C. Gunderson, Col., M.C.(Army)	Dept. of Neurology	Walter Reed Army Med. Center				J. Hallenbeck, CAPT., M.C.,(Navy)	Dept. of Neurology	Bethesda Naval Hosp.
Y. Leibowitz	Neuroepidemiology Unit	Jerusalem, Israel															
G. Palffy, M.D.,	Neurology, Univ. of Pecs	Pecs, Hungary															
C. Gunderson, Col., M.C.(Army)	Dept. of Neurology	Walter Reed Army Med. Center															
J. Hallenbeck, CAPT., M.C.,(Navy)	Dept. of Neurology	Bethesda Naval Hosp.															

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02424-08 NEB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Standardized Nomenclature and Coding of Neurologic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bruce S. Schoenberg, M.D., Dr.P.H. Vijay Chandra, M.D., Ph.D. Chief, Medical Officer, NEB, DIR, NINCDS NEB, DIR, NINCDS		
COOPERATING UNITS (if any) L. Kurland, M.D., Mayo Clinic, Rochester, MN; J.F. Kurtzke, M.D., Georgetown Univ., Washington, D.C.; F. Clifford Rose, M.B., FRCP, B. Benjamin, Ph.D., S. Haberman, M.A., FIA, and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England;*		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) To develop an internationally acceptable standard of nomenclature, classification, and coding of neurologic disorders.		
* Continued L. Schut, M.D., K. Kondo, M.D., Minneapolis, MN Toyko, Japan		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02570-05 NEB
PERIOD COVERED <u>October 1, 1986 through September 30, 1987</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Natural History of ALS-PD in Guam</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Bruce S. Schoenberg, M.D., Dr.P.H. Chief, Lawrence Lavine, DO, MPH, DTM & H Medical Officer,		NEB, DIR, NINCDS NEB, DIR, NINCDS
COOPERATING UNITS (if any) Steele, John M.D., Veterans Administration, Guam; Zhang, Xhen-xin M.D., People's Republic of China; Calne, Donald M.D., Univ. of British Columbia, Vancouver, BC; Spencer, P.Ph.D., Albert Einstein College of Medicine, NY		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 7.6	PROFESSIONAL: 1.6	OTHER: 6.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) As a continuation of previous projects on clinical, pathological, and epidemiologic surveillance of Guamanian amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia (PD) in the Mariana Islands, a total of 112 cases, including suspects registered as of January 1, 1983, are to be followed at intervals of six months for detailed clinical descriptions of patterns of progression by a qualified neurologist until all of the patients expire. A study of long surviving cases (over ten years) has been completed. Clinically they showed three patterns: (1) onset with slowly but steady progression at the same pace throughout the course; (2) rapid progression to complete paralysis of the limbs within 1.5 to 3 years and then remaining practically stable for the next 5 to 10 years; (3) onset with minimal atrophy and weakness for the first 5 to 6 years and then rapid step-wise progression to death. A significant number of PD cases were found to show not only lower motor neuron involvement but also severe pelvicurual flexion contractures in the advanced stage of the disease. This observation presents an important question of: (1) motor neuron involvement as a part of the natural history of chronic diseases of the CNS, or (2) a process identical to ALS which occurs in the same patient. If the latter is true, these cases may represent a continuum of ALS and PD, and thus indicate a single etiology of these two diseases. Prevalence survey of neurologic disease in addition to ALS and PD. The survey of ALS-PD is to document the potential decline in reported cases on Guam. Simultaneously a survey will be performed for other major neurologic diseases as this is a transitional society dietarily and culturally since WW-II.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02715-02 NEB																					
PERIOD COVERED October 1, 1986 through September 30, 1987																							
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Epilepsy Neuroepidemiology																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Bruce S. Schoenberg, M.D., Dr.P.H.</td> <td style="width: 30%;">Chief,</td> <td style="width: 30%;">NEB, DIR, NINCDS</td> </tr> <tr> <td>Lawrence Lavine, DO, MPH, DTM, & H</td> <td>Medical Officer,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Walter A. Rocca, M.D., M.P.H.</td> <td>Visiting Associate,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>P. Satish Chandra, M.D.</td> <td>Guest Researcher,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>Karin B. Nelson, M.D.</td> <td>Medical Officer,</td> <td>NEB, DIR, NINCDS</td> </tr> <tr> <td>James Dambrosia, Ph.D.</td> <td>Satistician,</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Wm. Theodore, M.D.</td> <td>Medical Officer,</td> <td>MNB, DIR, NINCDS</td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, DIR, NINCDS	Lawrence Lavine, DO, MPH, DTM, & H	Medical Officer,	NEB, DIR, NINCDS	Walter A. Rocca, M.D., M.P.H.	Visiting Associate,	NEB, DIR, NINCDS	P. Satish Chandra, M.D.	Guest Researcher,	NEB, DIR, NINCDS	Karin B. Nelson, M.D.	Medical Officer,	NEB, DIR, NINCDS	James Dambrosia, Ph.D.	Satistician,	BFSB, DIR, NINCDS	Wm. Theodore, M.D.	Medical Officer,	MNB, DIR, NINCDS
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Wm. Theodore, M.D.	Medical Officer,	MNB, DIR, NINCDS																					
COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J. F. Mellinger, M.S., M.R. Gomez, M.D., L.T. Kurland, M.D., Dr.P.H., and R.V. Groover, M.D., Dept. of Neurology, Mayo Clinic; L.L. Salkowicz, P. Gunerson, Ph.D., Minnesota Dept. of Health; F. Freifus, M.D., Charlottesville, VA.*																							
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research																							
SECTION																							
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.6	OTHER:																					
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The record-linkage system available for residents of Rochester, Minnesota has been used to identify all possible cases of complex partial, absence, and tonic-clonic seizures diagnosed between the years 1935 and 1979 who were born in the community. Case-control studies were conducted to identify risk factors associated with these seizure types. A study of the relationship of duration of untreated epilepsy to the ease or difficulty of its subsequent treatment is being carried out in several countries. The objective of the study is to determine how critical early diagnosis and early treatment are to the patient in terms of subsequent control of epilepsy. A case-control study of risk factors of intractability in complex partial seizures was conducted using clinic populations at several institutions. Publications for FY 1987 are listed with the NEB report. ----- *Continued <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Bahman Jabbari, M.D.</td> <td style="width: 60%;">Walter Reed Amry Medical Center</td> </tr> <tr> <td></td> <td style="text-align: right;">Washington, D.C.</td> </tr> <tr> <td>P. Ponce, M.D.</td> <td>Neurological Disease Caracas, Venezuela</td> </tr> <tr> <td></td> <td style="text-align: right;">Dept., Ministry of Health</td> </tr> </table>			Bahman Jabbari, M.D.	Walter Reed Amry Medical Center		Washington, D.C.	P. Ponce, M.D.	Neurological Disease Caracas, Venezuela		Dept., Ministry of Health													
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	Washington, D.C.																						
P. Ponce, M.D.	Neurological Disease Caracas, Venezuela																						
	Dept., Ministry of Health																						

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02746-01 NEB												
PERIOD COVERED October 1, 1986 through September 30, 1987														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Phenobarbital Clinical Trials in Children with Febrile Seizures														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Karin B. Nelson, M.D.</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 33%;">NEB, DIR, NINCDS</td> </tr> <tr> <td>Deborah Hirtz, M.D.</td> <td>Pediatric Neurologist</td> <td>DNB, NINCDS</td> </tr> <tr> <td>Young Jack Lee, Ph.D.</td> <td>Mathematical Statistician</td> <td>BFSB, DIR, NINCDS</td> </tr> <tr> <td>Jonas H. Ellenberg, Ph.D.</td> <td>Chief</td> <td>BFSB, DIR, NINCDS</td> </tr> </table>			Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINCDS	Deborah Hirtz, M.D.	Pediatric Neurologist	DNB, NINCDS	Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS
Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINCDS												
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Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, DIR, NINCDS												
Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINCDS												
COOPERATING UNITS (if any) Jacqueline Farewell, M.D., Dept. of Neurosurgery, Univ. of Washington, Seattle														
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research														
SECTION														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☒ (a) Human subjects</td> <td>☐ (b) Human tissues</td> <td>☐ (c) Neither</td> </tr> <tr> <td>☒ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☒ (a1) Minors			☐ (a2) Interviews					
☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither												
☒ (a1) Minors														
☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The objectives of the study are: To assess the effects on tests of intelligence and behavior of phenobarbital, commonly prescribed anticonvulsant in children, and to compare those effects with possible effects of recurrent seizures. The design of this study will permit comparison of measures of tested intelligence and of behavior in children with febrile seizures who have been treated with phenobarbital, and in a group of seizure-free control children. A comparison of the groups should allow assessment of benefit and risk of treatment in a common childhood neurologic problem.														

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02747-01 NEB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dental Markers of Maldevelopment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Karin B. Nelson, M.D.

Medical Officer,

NEB, DIR, NINCDS

COOPERATING UNITS (if any)

Mohandas Bhat, D.D.S., D.P.H., EODPP, NINDR

LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives are: To examine whether dental abnormalities, especially enamel defects, can serve as markers of maldevelopment, and whether such findings can provide information concerning timing of adverse events or exposures.

The significance of the research is that: Enamel hypoplasias and other dental anomalies can offer clues as to the timing of insults or exposures that occur from the fourth month of gestation to the age of about 12 months of extrauterine life. Correlation of dental with clinical data may offer a means to explore the timing of departure from the normal course of development in a group of children with chronic motor disability of early onset and nonprogressive course (cerebral palsy, or mental retardation).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02748-01 NEB
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Dermatoglyphic Markers of Maldevelopment		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINCDS		
COOPERATING UNITS (if any) Chris Plato, Ph.D., GRC, NIA		
LAB/BRANCH Neuroepidemiology Branch, Division of Intramural Research		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☒ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Fingerprints are in place by nine to 16 weeks of intrauterine life, dermal creases by 19 weeks. The objective of this pilot study is to examine whether dermatoglyphic abnormalities and dermal creases can serve as markers of maldevelopment, and whether such findings can provide information concerning timing of adverse events or exposures in children with abnormal neurologic development. The significance of the research is some reports of dermal abnormalities in children with early prenatal onset of maldevelopment. A time course of establishment of palmar creases has been developed. The hypothesis has been offered, and some data accrued to indicate, the dermal findings may offer clues as to the timing of insults or exposures during early prenatal life, providing a means to explore the timing of departure from the normal course of development in a group of neurologically handicapped children.		

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- Eldridge, R., Rocca, W.A., Ince, S.E.: Parkinson's Disease: Evidence Against a Toxic Etiology and for an Alternative Theory. In: Markey, S.P., Castagnoli, N. Jr., Trevor, A.J., Kopin, I.J. (Eds.), MPTP: A Neurotoxin Producing a Parkinsonian Syndrome. Academic Press, New York: 355-367, 1986.
- Eldridge, R.: Brain neurotransmitters in dystonia. N Eng J Med 316:279-280, 1987.
- Eldridge, R., Denckla, M.B.: The inheritance of Gilles de la Tourette syndrome. N Eng J Med 316:1347-1348, 1987.
- Bharucha, N.E., Stokes, L., Schoenberg, B.S., Ward, C., Ince, S., Nutt, J.G., Eldridge, R., Calne, D.B., Mantel, N., Duvoisin, R.: A case-control study of twin pairs discordant for Parkinson's disease: a search for environmental risk factors. Neurology 36:284-288, 1986.

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- Eldridge, R.: Clinical Neurogenetics. Camerini-Otero, D., Mulvihill, J.J., Schecter, A.N. (Eds.), Medical Genetics 1987. Foundation for Advanced Education in the Sciences, Bethesda: 430-447, 1987.
- Nee, L.E., Eldridge, R., Sunderland, T., et al.: Dementia of the Alzheimer type: CLinical and family study of 22 twin pairs. Neurology 37:359-363, 1987.

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Z01 NS 02299-11 NEB

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Schoenberg, B.S.: Clinical contributions of neuroepidemiology: prevalence, precursors, and prevention. Key Neurology and Neurosurgery 1 (4):8-11, 1986.

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Z01 NS 02301-11 NEB

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Haerer, A.F., Anderson, D.W., and Schoenberg, B.S.: Functional disability associated with major neurologic disorders. Arch. Neurol. 43:1000-1003, 1986.

Z01 NS 02715-02 NEB

Ellenberg, J.H., Hirtz, D.G., and Nelson, K.B.: Do seizures in children cause intellectual deterioration? New Engl J Med 314:1085, 1986.

Hirtz, D.G., Lee, Y.J., Ellenberg, J.H., and Nelson, K.B.: Survey on the management of febrile seizures. Am J Dis Child 140:909-914, 1986.

Nelson, K.B. and Ellenberg, J.H.: Antecedents of seizure disorders in early childhood. Am J Dis Child 140:1053-1061, 1986.

ANNUAL REPORT

October 1, 1986 through September 30, 1987

National Institute of Neurological and Communicative Disorders and Stroke

Neuroimmunology Branch

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Interaction Between Viruses and the Host Immune System Z01 NS 02205-12 NI	10
Molecular Mechanisms of Lymphoid Cell-Cell Interactions Z01 NS 02603-04 NI	11

Annual Report
October 1, 1986 to September 30, 1987
Neuroimmunology Branch
National Institute of Neurological and
Communicative Disorders and Stroke
Dale E. McFarlin, M.D., Chief

Research in the Neuroimmunology Branch (NIB) has continued to consist of both fundamental and clinical studies. Although the various research activities are interrelated and complementary, the Branch is divided administratively into four groups; The Office of the Chief, and three sections. The sections are Cellular Immunology, Molecular Immunology, and Neurological Diseases. During FY 1987, three major administrative changes occurred due to developments in the aims and objectives of the research in NIB: 1) The Neuropharmacology Section, which was established three years ago, was transferred to the Laboratory of Neural Regeneration and Implantation; 2) A Unit on Molecular Biology was established in the Neurological Diseases because of the increasing emphasis on genetics in immune regulation; 3) The Section on Molecular Immunology was established with Dr. William Biddison as Section Head.

Research in the Molecular Immunology Section is investigating the molecular basis for antigen recognition by immune cells. This research focuses on the T cell receptor (TcR) molecules involved in antigen recognition and the molecular nature of the target antigens recognized by TcR. A panel of DPw2-specific T cell clones previously developed by this group is being used for these studies. The diversity of the human TcR beta chain gene rearrangements and variable region gene usage has been analyzed in nine clones. Seven different clonotypes were detected, but three of the clones expressed the same V-beta gene (V-beta 14). The V-beta 14 gene was found to be rearranged in a significant portion of T cells in DPw2-specific bulk cultures from which the T cell clones were derived. These results suggest that, although the number of V-beta genes which contribute to a particular cell-mediated response (in this case a cytotoxic response to an alloantigen) may be relatively large, only a limited number of clonotypes ultimately predominate in the response to certain class II HLA antigens.

The research on the TcR germ-line V-beta gene repertoire has been extended to analysis of patients with multiple sclerosis (MS). The rationale for this research is based on the evidence that: 1) susceptibility to the disease is, in part, genetically controlled; and 2) certain animal models of disease characterized by demyelination such as experimental allergic encephalomyelitis (EAE) discussed below and Theiler's virus infection have been produced primarily in SJL mice. This strain has recently been shown to delete a significant portion of the germ-line V-beta repertoire. To date, DNA specimens from 38 MS patients has been analyzed with probes that detect 14 different V-beta subfamilies in collaboration with Drs. Leroy Hood and Patrick Concannon (Cal. Inst. Tech., Pasadena, California). No deletions of V-beta gene segments have been observed. The data indicate, however, that the distribution of V-beta gene haplotypes in patients with MS (as defined by

polymorphic alleles at V-beta 8, V-beta 11, and C-beta) differ significantly in MS patients when compared to the normal population. These preliminary findings suggest that there may be an MS susceptibility gene in the region of the TcR V-beta locus.

The CD8⁺ subset of T lymphocytes reacts with antigen in association with class I HLA molecules. The definition of the structural features that are important for T cell recognition of class I molecules has been pursued, using site-directed mutagenesis of a previously defined HLA-A3 variant and by isolating, sequencing, and manipulating additional T cell-defined variants of HLA-A2. Studies with HLA-A3 have shown that a single amino acid change (substitution of a valine for glutamic acid) at position 152 in the alpha 2 domain of the A3 molecule produces major structural changes in the epitopes that are recognized by class I-restricted virus-specific cytotoxic T lymphocytes (CTL). The importance of residues in the 147-160 region for class I-restricted CTL recognition was confirmed by finding changes in this region in two different HLA-A2 variant genes that have been sequenced. In addition, evidence was obtained that one of the HLA-A2 variant genes (M7A2.2) did not arise by a gene conversion-like event, because multiple substitutions were found in two different exons of the variant gene.

These fundamental studies performed in the Molecular Immunology Section are highly relevant to other research in the Branch. Several years ago the Cellular Immunology Section (CIS) demonstrated that measles virus-specific CTL are reduced in patients with MS. This is the only known antigen-specific defect in MS, and, a major emphasis of research in the CIS has been to examine this potentially important finding more extensively. Several additional avenues of research are in progress. These include studying the generation of measles virus-specific CTL in a large, well-defined MS patient population, examination of CTL responses to other viruses, examination of the generation of measles-specific CTL in subacute sclerosing panencephalitis (SSPE), and tropical spastic paraparesis (TSP). In addition, experiments that are designed to examine the T cell repertoire specific for the various measles virus polypeptides have been initiated.

The generation of measles virus-specific CTL has been studied in a population of well-defined group of MS patients during a phase of active progression. The findings from this large study have confirmed the previous observations that the measles virus-specific CTL response is significantly reduced in MS patients compared to healthy controls. Reduction of measles virus-specific CTL did not correlate with either degree of disability or HLA phenotype. Many patients with MS are DR2⁺, but reduction of measles virus-specific CTL in this group was not different from the non-DR2⁺ patients.

Measles virus-specific CTL are predominantly CD4⁺ T-cells that are restricted by class II HLA molecules. To determine if reduced measles virus-specific CTL in MS is a reflection of a more generalized abnormality of

class II-restricted CTL, the generation of class II-restricted CTL to mumps virus has been evaluated. Mumps-specific CTL are both CD4⁺ and CD8⁺ T-cells restricted by class I and class II MHC molecules, respectively. The portion of mumps CTL that is class II-restricted has been examined by using purified CD4⁺ T cells and measuring lysis of mumps virus-infected HLA class II matched targets. CD4⁺ T cells from MS patients with low measles virus CTL responses lysed mumps virus-infected targets in a normal manner, but failed to lyse the same targets when infected with measles virus. These studies indicate that reduction in measles virus-specific CTL is specific for measles virus and not simply a reflection of a generalized abnormality of CD4⁺ cells.

The generation of measles virus-specific CTL has been studied in patients with SSPE (a disease known to be related to latent or persistent measles virus infection) and in patients with TSP (a disease which has been recently shown to be associated with HTLV-I infection). The preliminary data indicate that patients with these disorders also have a defect in the generation of measles virus-specific CTL.

New concepts in the understanding of targets that are recognized by virus-specific CTL have important implications for this research. During virus infection, membrane components such as the measles HA protein are expressed on the surface of infected cells and are available for reaction with antiviral antibodies; however, recent studies from several laboratories, including the NIB, have shown that intact surface molecules are not the exclusive target of immune cells. The findings indicate that CTL recognize processed antigen co-expressed with components of the MHC and that so-called "internal" viral components such as nucleocapsid (N) protein are recognized by some virus-specific CTL. This implies that internal polypeptides are processed, transported to the cell surface and co-expressed with appropriate MHC molecules. This concept has major implications for assessment of cellular immunity, not only to viral antigens, but other components of the nervous system such as autoantigens.

The cellular immune response to individual measles virus polypeptides is being pursued using two strategies. Measles virus polypeptides are being purified by affinity chromatography and used to generate specific CTL. Secondly, major emphasis is being placed on establishing tissue culture cell lines that are co-transfected with HLA class II genes and genes for individual virus polypeptides. This undertaking has been successful by employing genes that encode the matrix (M) and N proteins. Murine fibroblasts co-transfected with HLA class II genes, as well as M or N genes have been produced. These transfectants can be lysed by measles virus-specific CTL. Because M and N proteins are "internal" components of measles virus, the findings support the concept that measles virus-specific CTL can recognize internal viral components. Messenger RNA for M and N is present in the transfected cells, but intact polypeptides have not been demonstrated using anti-measles antisera. These observations imply that the measles virus polypeptides are

processed after synthesis and that the epitopes recognized by T cells are different from those recognized by antibodies.

The mechanisms responsible for entry of immune cells into the central nervous system (CNS) and for production of an inflammatory response in the CNS are being studied using chronic-relapsing EAE in mice. In the past, experiments conducted in collaboration with Dr. Maria Spatz in the Laboratory of Neuropathology and Neuroanatomical Sciences have shown that normal murine CNS endothelial cells (EC) lack both class II MHC molecules and the capacity to function as antigen-presenting cells. However, after treatment with gamma interferon, class II MHC molecules are expressed on EC that then acquire the capacity to present antigen to sensitized immune cells. Recent findings indicate that this property varies in EC from different mouse strains. Our initial studies were conducted in SJL mice (H-2^S) that are susceptible to EAE. Recently, it has been observed that EC from Balb/c mice (H-2^d) that are relatively resistant to EAE do not express class II MHC molecules after incubation with gamma interferon. This suggests that expression of class II MHC molecules may correlate with susceptibility to EAE. Further, antigen presentation in immune organs and in the CNS may also vary, because no differences were observed in the antigen-presenting functions of splenic macrophages from Balb/c mice. The conclusions from these experiments may also apply to other CNS cells such as astrocytes that can function as antigen-presenting cells.

During the course of chronic-relapsing EAE, the phenotype of cells in the CNS lesions change. Initially, the lesions are largely composed of Lyt-1⁺ cells but, in time, the other phenotype, Lyt-2⁺, increases. Methods have recently been developed to establish T cell lines and clones from the CNS of mice with the chronic disease. This should facilitate functional characterization of the cells derived from lesions in the CNS.

Most studies of chronic-relapsing EAE have been conducted in SJL mice, and it is of considerable interest that two significant immunological differences have been observed in SJL mice as well as certain other murine strains. H-2^S mice express only I-A but not I-E class II MHC molecules and have a deletion of approximately 50 percent of the germ-line V-beta T cell receptor gene segments. Although these abnormalities are of considerable interest and may relate to the pathogenesis of chronic-relapsing EAE, it is important to assess EAE in other strains. Consequently, chronic EAE produced by adoptive transfer of immune cells is being evaluated in PL mice. In initial pathological studies in this strain, large necrotic lesions, infiltrated by eosinophils, were found in the white matter. Some areas also showed budding virions resembling a corona virus. Analysis of sera from these animals showed antibodies to murine hepatitis virus (MHV). Because of the possible contribution of a viral agent to the relapsing nature of the disease and to the pathology, the experiments were repeated using mice free of MHV infection and maintained in a germ-free environment. These virus-free mice developed chronic EAE that was clinically indistinguishable from that seen in SJL mice.

However, the pathology consisted of primary demyelination similar to that previously observed in SJL mice, and these experiments illustrate that interaction between an autoimmune process and a latent virus infection can markedly modify the underlying pathology in the nervous system in the absence of significant clinical changes.

During the past year, results of studies performed in collaboration with Drs. B. Roy and D. Murphy in NIMH on a group of patients with affective disorders who have antibodies that react with beta endorphin were reported. The anti-beta endorphin has been isolated by affinity chromatography and used to seek evidence of an anti-idiotypic in the serum of patients with the disorder. Preliminary data indicate the presence of immunoglobulins which can block the binding of antipeptide endorphin to the peptide. The findings also indicate that the putative anti-idiotypic can compete with radiolabelled ligand for the opioid receptor. These findings have led to the speculation that immune networks consisting of anti-idiotypes directed at antipeptide antibodies operate in parallel to the traditional neuroendocrine systems.

Clinical research in the NIB continues to be focused on investigation of MS, but patients with other important diseases are also being studied. These include Sjögren's disease in collaboration with Dr. Elaine Alexander, (Johns Hopkins), SSPE in collaboration with Dr. John Sever, (IDB), and TSP associated with HTLV-I infection in collaboration with Drs. Clarence J. Gibbs, Jr. and D. Carleton Gadjusek, (LCNRS). The studies on MS involve the assessment of clinical, neuroradiological, and immunological findings in patients with newly diagnosed disease, the clinical and immunological assessment of families in which there is more than one affected individual, and the evaluation of cyclosporine A in a double-blind randomized trial. Specimens from these groups of patients are widely used by many investigators in the Branch. These include fundamental investigations of cellular function, immunogenetic background, and genes coding for the T cell repertoire. For such fundamental studies to be relevant, well-defined clinical material is essential.

The double-blind randomized trial of cyclosporine A has entered the final year. Approximately 20 patients have withdrawn from the study, mainly because of the progression of their disease. Since the study is being conducted under code, it is not known if these individuals were on placebo or cyclosporine A. It is anticipated that the study will be completed by January of 1988. Because cyclosporine A is nephrotoxic and because the unblinded observer in the double-blind randomized trial has detected some degree of renal dysfunction in some patients, at the conclusion of the cyclosporine A trial, patients are undergoing an extensive renal evaluation in collaboration with Dr. Howard Austin of the Clinical Center. Subsequently, all patients are given the opportunity to receive cyclosporine A in an open manner until the efficacy of the drug can be either established or refuted by analysis of the overall findings. The immunological parameters of these patients will continue to be studied in a longitudinal manner.

Clinical, immunological, and virological studies of TSP have recently been initiated. This disease has been demonstrated to be associated with increased antibodies in the serum and spinal fluid to HTLV-I, and it has been postulated that this human retrovirus directly causes the neurological disorder. To date, four patients: two from Colombia, one from Haiti, and one from Jamaica have been studied. All four have high titers of antibody to HTLV-I, and it is of particular interest that three have MRI abnormalities in the periventricular and cerebellar white matter that are similar to those found in patients with classical MS. Because some human retroviruses can infect lymphocytes and alter immunological function, extensive evaluation of the blood and CSF lymphocytes is being conducted. Significant numbers of large lymphocytes that have been activated in vivo have been observed in the blood of all patients studied. These cells express DR molecules and IL2 receptors which indicates lymphocyte activation. In addition, lymphocytes from these patients undergo spontaneous proliferation in tissue culture. Collectively, the data demonstrate that significant changes consistent with the known in vitro effects of HTLV-I have occurred in lymphocytes of these patients. Consequently, extensive efforts have been initiated to detect a retrovirus genome in lymphocytes from these individuals. It is obviously important to conduct parallel studies in patients with other neurological diseases, and particularly, in MS. Retroviruses are known to produce chronic neurological diseases in animals and it has been postulated that such agents are candidates for the etiology of CNS diseases in humans. Assessment of immune function in such disorders could provide data to support this possibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02202-12 NI
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunological Studies in Patients with Multiple Sclerosis and other CNS Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Dale E. McFarlin, M.D.	Chief	NI DIR NINCDS
Others: Henry F. McFarland, M.D.	Deputy Chief	NI DIR NINCDS
Andrew Goodman, M.D.	Medical Staff Fellow	NI DIR NINCDS
Steven Jacobson, Ph.D.	Senior Staff Fellow	NI DIR NINCDS
Lauren Krupp, M.D.	Medical Staff Fellow	NI DIR NINCDS
Ajay Gupta, M.D.	Visiting Associate	NI DIR NINCDS
David Mattson, M.D.	Medical Staff Fellow	NI DIR NINCDS
Jack Strominger, Ph.D.	Professor	Harvard University
COOPERATING UNITS (if any) Department of Biochemistry and Molecular Genetics, Harvard, Boston		
LAB/BRANCH Neuroimmunology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 12.0	PROFESSIONAL: 8.0	OTHER: 4.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Investigation of patients with neurological dysfunction. The general aim of this project is to obtain a more precise understanding of the multiple factors possibly related, either singly or in combination, to the pathogenesis of neurological disorders. Focus is currently placed on multiple sclerosis (MS). Patients with sporadic disease, patients with a family history of demyelinating disease, as well as identical and nonidentical twins who are either concordant or discordant for the disease are being evaluated. The studies include assessment of immunogenetic background, cellular immune reactivity to a number of viruses and cell surface molecules on leukocytes in the blood. The reactivity and production of immunoglobulins in the cerebrospinal fluid are being measured by highly sensitive techniques. These investigations have recently been extended to tropical spastic paraparesis (TSP) associated with HTLV-I. Magnetic resonance imaging is being used to assess the extent and magnitude of lesions in MS and TSP. A phase III cooperative trial of cyclosporine A in chronic-progressive MS is currently in progress. During this trial clinical status, findings by magnetic resonance imaging and immune status are being followed.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02204-12 NI
PERIOD COVERED October 1, 1986 through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Mechanisms Operative in Experimental Autoimmune Diseases of the Nervous System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Dale E. McFarlin, M.D. Others: Richard McCarron, Ph.D. Anne Cross, M.D. Robert Fallis, M.D. Ute Traugott, M.D. Cedric Raine, Ph.D. G.R. Wayne Moore, Ph.D. Maria Spatz, M.D.	Chief Sr. Staff Fellow Research Fellow Sr. Staff Fellow Assistant Professor Professor Research Fellow Section Head	NI DIR NINCDS NI DIR NINCDS Albert Einstein U. NI DIR NINCDS Albert Einstein U. Albert Einstein U. Albert Einstein U. LNNS DIR NINCDS
COOPERATING UNITS (if any) Departments of Pathology (Neuropathology) and Neuroscience, Albert Einstein College of Medicine, New York, N.Y. LNNS, NINCDS		
LAB/BRANCH Neuroimmunology		
SECTION Neurological Diseases Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The mechanisms responsible for the production of experimental allergic encephalomyelitis (EAE), a model of autoimmune disease which is manifested by demyelination, are being examined. Current research is focused on an adoptively transferred model which is produced by the transfer of lymphocytes sensitized against myelin basic protein in syngeneic animals. Under optimal conditions, neurological dysfunction occurs; this is characterized pathologically by inflammation and primary demyelination. Many mice recover and develop chronic-relapsing disease. The mechanisms responsible for both the initial and the chronic disease are not known, but an early event is the migration of immune cells across the blood-brain barrier into the central nervous system (CNS). The subpopulation of T-lymphocytes which is responsible for the disease are Lyl⁺2⁻ and react with antigen in association with class II MHC (Ia) molecules. Immunohistochemical examination of CNS endothelial cells (EC) in tissue section and the cytofluorographic studies of whole cells freshly isolated from the CNS indicate class II MHC molecules are not expressed on normal EC, but appear during the development of EAE. Immune lymph node cells with the capacity to transfer EAE produce gamma interferon which can induce the expression of Ia molecules on EC cells. These observations have led to the hypothesis that interaction between immune cells and the capillary endothelium leads to alterations of the blood-brain barrier. EC have been isolated from mice with acute disease. Some of the cells are Ia⁺ and can present antigen. The mechanism responsible for the induction of Ia molecules and the genetics of Ia expression is being studied <u>in vitro</u> in strains of mice that are susceptible and resistant to EAE.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02603-04 NI																																								
PERIOD COVERED October 1, 1986 through September 30, 1987																																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Mechanisms of Lymphoid Cell-Cell Interactions																																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 45%;">PI: William E. Biddison, Ph.D.</td> <td style="width: 25%;">Section Chief</td> <td style="width: 10%;">NI</td> <td style="width: 10%;">DIR</td> <td style="width: 10%;">NINCDS</td> </tr> <tr> <td>Others: Mary L. Jelachich, Ph.D.</td> <td>Special Volunteer</td> <td>NI</td> <td>DIR</td> <td>NINCDS</td> </tr> <tr> <td>Elli Leontsini, M.D.</td> <td>Special Volunteer</td> <td>NI</td> <td>DIR</td> <td>NINCDS</td> </tr> <tr> <td>Steven Beall, M.D.</td> <td>Medical Staff Fellow</td> <td>NI</td> <td>DIR</td> <td>NINCDS</td> </tr> <tr> <td>Elliot Cowan, Ph.D.</td> <td>Senior Staff Fellow</td> <td>NI</td> <td>DIR</td> <td>NINCDS</td> </tr> <tr> <td>John Coligan, M.D.</td> <td>Section Chief</td> <td>LIG</td> <td>DIR</td> <td>NIAID</td> </tr> <tr> <td>David Nelson, M.D.</td> <td>Senior Investigator</td> <td>MET B</td> <td>DIR</td> <td>NCI</td> </tr> <tr> <td>David Mattson, M.D.</td> <td>Medical Staff Fellow</td> <td>NI</td> <td>DIR</td> <td>NINCDS</td> </tr> </table>			PI: William E. Biddison, Ph.D.	Section Chief	NI	DIR	NINCDS	Others: Mary L. Jelachich, Ph.D.	Special Volunteer	NI	DIR	NINCDS	Elli Leontsini, M.D.	Special Volunteer	NI	DIR	NINCDS	Steven Beall, M.D.	Medical Staff Fellow	NI	DIR	NINCDS	Elliot Cowan, Ph.D.	Senior Staff Fellow	NI	DIR	NINCDS	John Coligan, M.D.	Section Chief	LIG	DIR	NIAID	David Nelson, M.D.	Senior Investigator	MET B	DIR	NCI	David Mattson, M.D.	Medical Staff Fellow	NI	DIR	NINCDS
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The general objective of this project is to define the mechanisms by which human lymphoid cells interact with each other and with foreign antigen-bearing cells in order to produce and regulate immune responses. Over the past year, there have been two major efforts underway that are targeted on this objective: 1) dissection of the molecular basis for T cell recognition of class I HLA gene products; and 2) analysis of human T cell receptor gene usage in alloreactive T cell populations. The definition of the structural features of class I HLA molecules that are important for T cell recognition has been pursued using site-directed mutagenesis of a previously defined HLA-A3 variant and by isolating, sequencing, and manipulating additional T cell-defined variants of HLA-A2. The results of these studies demonstrate that a single amino acid change (substitution of valine for glutamic acid) at position 152 in the alpha 2 domain of the A3 heavy chain produces major structural changes in the epitopes that are recognized by both HLA-restricted and alloreactive cytotoxic T lymphocytes (CTL). The importance of residues in the region 147-160 for HLA-restricted CTL recognition was confirmed by the findings of amino acid changes in this region in two different HLA-A2 variant genes (M7A2.2 and DK1A2.3) that we have isolated and sequenced. Analysis of the diversity of T cell receptor beta chain gene usage indicates that although a large number of V-beta genes can be used to recognize a particular allospecificity, a limited number of V-beta genes will ultimately predominate in the response.																																										

ANNUAL REPORT
October 1, 1986 through September 30, 1987
Surgical Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke
Edward H. Oldfield, M.D., Chief

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National Institute of Neurological and Communicative Disorders and Stroke

I. SUMMARY OF STUDIES

The Surgical Neurology Branch (SNB) principal activities are conducted in the following: 1) Clinical Neurosurgery Section; 2) Biochemistry Unit; 3) Central Nervous System Implantation Unit; 4) Tumor Biology Unit; 5) Neuroimmunology Unit; 6) Electron Microscopy Unit; and 7) Cellular Biology Unit.

The SNB has as its major research functions: (1) the study of the biology and therapeutic approaches to malignant and benign tumors of the brain, and pituitary gland; (2) investigation of the capability of implanted tissues into the brain of laboratory animals and humans to survive and integrate anatomically or biochemically with the host brain to influence brain function; (3) the investigation of certain vascular disorders of the CNS; and (4) the investigation and surgical treatment of patients with epilepsy. Its clinical function is to provide the neurosurgical services to its own research protocol patients and to patients seen in consultation in the NIH Clinical Center. The SNB is presently located in Building 10A, 10 and 9. Its staff includes 15 clinical neurosurgeons at various levels of training and experience, as well as 1 senior scientist, 3 junior scientists and a support staff of technical and administrative individuals.

In addition to its primary functions of clinical and basic research, the SNB provides young neurosurgeons with experience in clinical and laboratory investigation in a combined clinical and neuroscience environment. Of those who participate in the SNB program as medical staff fellows and senior staff fellows, many have entered or are planning to enter academic roles in neurosurgery.

The following significant observations have been made during the past 12-18 months. In the area of brain tumors we have made several observations. Drug streaming, which is responsible for toxicity and limitation in efficacy after intraarterial infusion of chemotherapy, can be eliminated by phased diastolic infusions using a specially designed infusion pump. Using intraarterial infusion of cisplatin combined with extracorporeal circulation of the jugular venous blood, nearly complete vascular isolation of the brain was achieved. Therefore, the cerebral circulation can be isolated by the rather simple means of two transcutaneous catheters, one in the carotid artery and the other in the ipsilateral jugular vein. Extracorporeal hemodialysis of the ipsilateral jugular blood removes as much as 90% of the cisplatin infused into the internal carotid artery before this drug reaches the systemic circulation and sensitive normal tissues are exposed.

A protein that is secreted by malignant brain tumors and which increases vascular permeability to macromolecules has been isolated from the incubation media from malignant brain tumors. We have demonstrated that this material is a protein of approximately 45,000 daltons molecular weight and that its secretion is eliminated by incubation by the tumor cells with dexamethasone. It was also shown that, using the PET scan and gallium-EDTA to study patients with malignant brain tumors before and after steroid therapy, diminished interstitial fluid volume was demonstrated in response to the treatment with dexamethasone.

Using PET scanning and fluoro-deoxy-glucose thiopental and pentobarbital, we demonstrated to selectively suppress brain metabolism by approximately 50% while leaving tumor metabolism unaffected. Similar, but less suppression, was demonstrated with sedative doses of pentobarbital. Means to exploit this capacity to selectively influence the brain metabolism without effecting tumor metabolism for therapy are currently under investigation. This technique is also a technique of PET "contrast enhancement" and should be applicable to enhance the demonstration of other, non-tumorous, lesions of the brain.

Adoptive immunotherapy with IL-2 and lymphokine activated killer cells has demonstrated that IL-2 alone prolongs survival of a model of brain tumor in rodents. However, experience in the laboratory and in the clinic demonstrate that IL-2 induces a vascular leak syndrome which affects tumor vessels and greatly enhances cerebral edema surrounding brain tumors. Electron microscopy demonstrated that the interaction of lymphokine activated killer cells with malignant brain tumor cells, which produces cytotoxicity, was based on the local exocytosis of granules or vesicles by the effector cell into the small extracellular space between the effector/target cell conjugate. This is a common cytotoxic mechanism for cytotoxic T cells, natural killer cells, and LAK cells against tumor cell targets. Techniques were perfected for successful and reproducible implantation of dispersed LAK cells into the brain of host animals. This information was used to treat patients with direct inoculation of the lymphokine activated killer cells and IL-2 into malignant brain tumors. Repeated injections of lymphokine activated killer cells and IL-2 into the cavities of cystic malignant brain tumors elicited partial tumor responses in 2 of 6 patients, but increased cerebral edema of clinical significance in all patients treated.

An antitransferrin antibody-toxic peptide conjugate was created in the transferrin receptor which specifically kills tumor cells in vitro and also when injected into the cerebrospinal fluid. Animal studies indicated a 5-log kill of tumor cells inoculated into the peritoneal cavity in vivo. Treatment of tumor in a guinea pig model of leptomeningeal neoplasia demonstrated a 2 to 3-log, and in some animals a 5-log, kill of tumor cells in the central nervous system with this conjugate. A cloned diphtheria toxin was created in which the C-terminal 15KD region was deleted and linked to MoAb. Recent results indicate that these point mutations can increase selective cytotoxicity of antibody-toxin conjugates up to 10,000-fold.

Further experience with selective pituitary venous sampling for Cushing's syndrome indicated that selective bilateral simultaneous inferior petrosal sinus sampling successfully distinguishes patients with Cushing's disease from those with ectopic ACTH syndrome with greater than 99% accuracy. This technique permits tumor localization within the pituitary gland in patients with Cushing's disease with about 85% accuracy.

Retrospective assessment of 81 patients with spinal AVMs indicated that there are 2 major types of AVMs affecting the spine, arteriovenous fistulas and true AVMs of the spinal cord. Distinct clinical syndromes were identified to be associated with each of these types of spinal AVMs, different mechanisms of the production of myelopathy were clearly identified, and demographic considerations indicate that spinal dural AV fistulas are acquired, rather than congenital in origin.

The investigation of the mechanism and physiological role of programmed cell death, which occurs in both the central nervous system and the immune system during normal development, has demonstrated that thymocytes could be induced to die by the same signal that stimulates mature T cells to proliferate. RNA and protein synthesis inhibitors which block new gene expression interrupted the programmed cell death. This is consistent with a new model of immune system self tolerance that states the potentially autoreactive cells are induced to die by crosslinking the T cell receptor early in development, the same signal that induces mature T cells to react against foreign antigens.

Encouraging progress was made in the area of implantation of tissues from the central nervous system into the host brain in primates. Initially, a model of Parkinsonism which affects only one-half of the brain was developed by infusing MPTP into the internal carotid artery. Using this model of hemi-Parkinsonism and the previously available model of MPTP-induced full Parkinsonism in the primate, we investigated functional recovery and graft survival after implanting dopaminergic tissues into the caudate nucleus of lesioned animals. Both adrenal and fetal substantia nigra allografts were demonstrated to induce partial functional recovery in the host animals. However, the recovery was much more pronounced using fetal substantia nigra for implantation. Immunohistological assessment demonstrated functional survival of the fetal grafts and prominent sprouting of dopaminergic fibers from resident fibers in the host brain surrounding the area of implantation. These results indicate that sprouting of the intrinsic dopaminergic pathways of the host brain is induced by either the surgical cavitation or the fetal implants. If the basic mechanisms responsible for this induced sprouting can be identified and used for other neuronal systems, practical application of central nervous system implants for Parkinson's disease and other CNS disorders may be possible.

A. CLINICAL NEUROSURGERY SECTION

Edward H. Oldfield, M.D., Chief

The clinical activities of the Surgical Neurology Branch are primarily directed to the investigation of the biological behavior and mechanisms of pathophysiology of malignant primary brain tumors, pituitary tumors, certain vascular disorders of the CNS, the surgical management of epilepsy refractive to medical therapy, and the investigation of tissue implantation in the CNS to reverse neurological disorders.

Primary Brain Tumors

1. Intraarterial Chemotherapy - Drs. Edward Oldfield, Stephen Saris, Donald Wright

One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to a drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intracarotid infusion. Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high concentration of drug reaches the general circulation, should allow dose escalation to levels which provide increased tumor response. We have developed techniques which provide regional vascular isolation of a tumor-bearing region by percutaneous catheterization of the afferent and efferent vessels. This permits the venous drainage of the region to be circulated extracorporeally for drug removal before the high concentration of drug reaches the systemic circulation. The following have been demonstrated in the past year.

- a. Drug streaming during intracarotid delivery results in maldistribution of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas received minimal drug. Patients who received intracarotid BCNU were studied with CT scanning, PET scanning, and MRI; the results correlated with histopathology to demonstrate that 1) progressively enlarging cerebral lesions which are often seen on CT and MRI scans following intracarotid chemotherapy may not be tumor recurrence but sites of focal cerebral necrosis; and 2) drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU.
- b. Drug streaming was studied in rhesus monkeys by comparing the distribution of the deposition of ^{14}C -iodoantipyrine during two methods of intracarotid infusion. A rapid retrograde infusion eliminated the prominent heterogeneous distribution of drug deposition which occurred during the slow infusion (the slow infusion was at a rate analogous to that which is being commonly used clinically). This study strongly suggests that current methods of intraarterial drug delivery, to the brain and other sites, are associated with an unpredictable and variable drug distribution and that this maldistribution can be eliminated by

techniques such as a rapid infusion, which eliminate intraarterial streaming.

- c. In experiments recently completed in the DRS by Drs. Shook, Dedrick, Lutz and Dopman, drug streaming was eliminated in an in vitro model of the human carotid arterial system by pulsed intraarterial infusion during diastole. This was evaluated by Dr. Stephen Saris in rhesus monkeys with pulsed diastolic injection of labeled ^{14}C -iodoantipyrine into the internal carotid artery. The pulsed diastolic injection is performed with a special pump that is linked to the cardiac cycle so that infusate enters the vessel during the diastolic portion of the flow in the parent vessel. Results using quantitative autoradiography to quantify drug delivery in rhesus monkeys suggest that this technique will allow intraarterial drug delivery with elimination of drug streaming, and will do so at rates of infusion that can be conveniently used clinically. The presence of streaming, and its elimination by phased diastolic diffusion, are being investigated in vivo using the PET scan and a short half-life positron emitting isotope of oxygen, ^{15}O . Evaluations are underway in subhuman primates and in clinical studies during intracarotid infusions of chemotherapy in humans. We are now using this system to deliver cisplatin (100 mg/m^2) into the internal carotid artery of humans and using the PET scan with ^{15}O infusion to assess drug streaming and its elimination with phased diastolic delivery.
- d. Since small laboratory animals are often used for pilot experiments in antitumor drug therapy, and in experiments in the areas of pharmacology, physiology and biochemistry, it was important to know if drug streaming occurred after intraarterial infusion into the small arterial systems of these animals. Dr. Stephen Saris, using quantitative radiography, demonstrated that after intracarotid infusions using conventional techniques in the rat, maldistribution of the isotope delivery into the profused brain tissue was a serious problem and that it could be eliminated by fast retrograde infusion. This important observation suggests that if the technique of intraarterial infusion in small laboratory animals is not carefully considered, heterogeneous distribution of the infused agent may lead to invalid or misleading results.
- e. A preliminary study, performed in vitro, demonstrated that about 90% of the cisplatin in whole blood circulating at 300 ml/min could be removed by hemodialysis using 2 hemodialysis cartridges in series. We then treated 4 patients with cisplatin by intracarotid infusion of a dose that is widely used intravenously. Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion effected or generated tumor exposures 5-15 fold greater than the exposure of the remainder of the body. We then performed 4 treatments in humans using high-dose cisplatin (200 mg/m^2) combined with drug removal by extracorporeal hemodialysis. The results of the systemic drug levels suggest that the body was exposed to less than 1/2 of the exposure expected if the drug-removal technique had not been used. However, retinal and brain toxicity was intolerable.

2. Investigation of Cerebral Edema with Brain Tumors

Brain edema frequently determines the degree of neurologic dysfunction in many disease states. The successful management of vasogenic edema by corticosteroids has had a major impact in the management (perioperatively; during administration of radiation therapy) and quality of survival in patients with malignant brain tumors. The mechanism of action of steroids in the treatment of vasogenic edema is not known; an understanding may optimize the use of steroids in this pathologic state and lead to new treatment methods for various forms of brain edema.

a. ⁶⁸Gallium-EDTA PET Scanning in Humans with Malignant Brain Tumors - Don Wright, M.D.

Positron scanning using labelled EDTA is a means to examine brain edema, vessel permeability patterns, and the mechanisms of action of drugs used to treat brain edema. EDTA distributes in the brain extracellular space in a pattern analogous to large water soluble molecules (e.g., is largely excluded from the normal brain). The PET scan can be employed to indirectly determine permeability in pathologic states by measuring the flux, or clearance (transfer constants K_1/K_2) of the labelled EDTA from normal and pathologic areas. In addition, the volume of the extracellular space can be determined by dynamic PET scanning. One of the mechanisms of action of dexamethasone is thought to occur by an effect on the volume of the brain extracellular space. The effects of dexamethasone on the edema/permeability patterns were examined in six patients with metastatic brain tumors using paired PET scans before and after treatment with dexamethasone. The general permeability patterns were further examined in six additional patients with primary malignant brain tumors. Patients with metastatic tumors had minimal changes in permeability when given high dose dexamethasone, whereas the volume of the extracellular space decreased 15-20% after the dexamethasone.

b. Elaboration of a Factor by Malignant Gliomas Which Increases the Permeability of Normal Blood Vessels In Vivo - Drs. Gregory R. Criscuolo, Marsha Merrill, Edward Oldfield

One of the pathophysiological mechanisms of the production of an intracranial mass effect by primary and secondary malignant tumors is by the tumor eliciting cerebral edema in the surrounding normal tissue. We have demonstrated that media from malignant gliomas in monolayer cultures secrete a substance which, when injected intradermally into guinea pigs, increases the accumulation of a circulating radioisotope (I^{125} RISA) and a marker dye (Evan's blue) at the site of injection compared to media from fibroblasts, benign brain tumors, normal saline and tissue culture media. The production of the increased vascular permeability factor in the media from malignant gliomas can be abrogated by incubation of the tumor cells with dexamethasone and by inhibition of protein production by cyclohexamide. Partial characterization of the factor suggests that it is a 35,000-45,000 molecular weight protein. (See Tumor Biology Laboratory see page 24.)

3. PET Scanning While Using Barbiturate Anesthesia to Accentuate the Difference in Glucose Metabolism of Brain Tumors and Normal Brain

This project investigates FDG-PET scanning of patients who have brain tumors before and during barbiturate anesthesia. The results using coma-producing doses of barbiturates indicated that a profound reduction of cerebral glycolytic activity can be achieved with a level of anesthesia which produces burst-suppression EEG activity. Gliomas, however, have only a minimal change in glycolytic rate under barbiturate anesthesia. The results also indicate that some lesions, which are not visible on PET scans performed with the patient awake, become visible as background synaptic activity is suppressed with barbiturates. The true extent of growth into the surrounding tissue can be better appreciated when background activity is reduced under the barbiturate anesthesia. Since coma-inducing doses of barbiturates would be inconvenient for clinical use, we investigated the possibility of achieving a portion of this effect using sedative doses of pentobarbital. This resulted in the demonstration that about fifty percent of the suppression in glucose metabolism by normal brain using coma-producing doses of barbiturates can be achieved by pre-anesthetic, sedative, doses of pentobarbital. Therefore, using doses of pentobarbital that could be used repeatedly and conveniently in the clinic we have achieved suppression of brain metabolism while leaving tumor metabolism unaffected. This work provides evidence that the use of barbiturates during FDG-PET scanning may allow a "reverse contrast enhancement" of lesions with decreased neuronal activity. This phenomenon may provide the basis for development of specific antitumor therapy. For instance, if a portion of the dose limiting toxicity of an antitumor therapy is related to the presence of synaptic activity then the dose of that agent might be safely increased during elimination of neural transmission using barbiturate treatment simultaneously. Since the most effective therapy available for malignant brain tumors is radiation therapy we initially investigated the capacity of barbiturates as protective agents during radiation treatment of rats. The results of the first set of experiments demonstrate that there is a striking protection of the radiation induced brain toxicity by the administration of pentobarbital just before exposure to the ionizing radiation. In further studies we are now investigating the mechanism of this protective effect. This PET technique also may prove to be valuable in studying other pathological processes such as degenerative diseases, epilepsy, movement disorders, cerebral infarction and head injury.

4. 125 Iodine Interstitial Brachytherapy for Primary Malignant Brain Tumors. A Phase I-II Study - Don Wright, M.D.

Interstitial radiation is increasingly being applied to primary malignant brain tumors (MBT) to deliver high local doses of radiation to a disease which is largely a regional problem. Efficacy has been demonstrated, although no consensus has been reached on the total dose, dose rate, treatment volume and

isotope characteristics best suited for these tumors. This study was developed in collaboration with the NCI Radiation Oncology Division to address the effects of high-dose interstitial radiation on normal and pathologic tissue using a variety of dose rates and total doses. Patients were divided into three groups with a range of initial dose rates (15, 20 and 30 cGy/hr) and total doses (100-280 Gy) using a homogeneously distributed interstitial approach. Twenty evaluable patients with malignant brain tumors (glioblastoma multiforme or anaplastic astrocytoma) were treated with semi-permanent 125I implants using image-directed stereotaxis for placement of multiple source arrays. Patients with recurrent tumors previously treated (5 patients) received only an interstitial boost, while the primary treatment group (15 patients) received whole brain radiation (45 Gy) plus an interstitial boost. Patients did not receive any other adjuvant therapy following an implant.

Ten of the twenty patients remain alive. Survival times range from 2-32 months following an implant, and 5-37 months following diagnosis. There were no surgical mortalities; morbidity from surgery arose from 3 complications (one hemiparesis; two local infections). Early radiation toxicity (within 2 months) was limited to radiation-induced peritumoral edema (8 patients) and was observed only in those patients with lesions greater than 4cm in diameter. Late radiation effects (greater than 2 months) observed by scanning studies typically demonstrated decreased enhancement of the tumor region, decreased mass effect, atrophic changes in surrounding brain and calcification in areas immediately adjacent to the radiation sources. Fluoro-deoxyglucose PET studies (six patients) demonstrated focal necrosis corresponding to the treatment volume surrounded by a zone of decreased cerebral metabolism. Karnofsky scores were sustained at the preoperative level in eight patients, fell by 10 points in six patients, and by 20 points in two patients.

Pathologic exam of tissue was performed in seven patients. Three were subjected to surgical procedures: residual tumor was found outside of the implant volume in two patients, and radiation necrosis without tumor occurred in one patient. Four patients had autopsies with one persistent tumor in the implant volume, one with tumor adjacent to the implant volume, two with necrosis only, and one patient with a contralateral tumor developing 16 months after a diagnosis.

Conclusions of the current study are 1) the interstitial approach developed has acceptable surgical and radiation risks with respect to early toxicity, and 2) the use of multiple arrays of low activity sources to distribute the radiation dose has a lower incidence of symptomatic radiation necrosis, 3) smaller treatment volumes have fewer toxic reactions regardless of the dose rate and total dose, and 4) no conclusions can yet be reached regarding the best dose rate and total dose.

5. 2-Deoxy-D-glucose Studies in the 9L Intracerebral Rat Model - Don Wright, M.D.

Malignant cerebral tumors utilize more glucose than normal brain, and therefore may be susceptible to metabolic agents which interfere with glucose

metabolism. Pharmacologic doses of 2DG might disrupt glucose utilization sufficiently to selectively retard tumor metabolism. In prior investigations we have shown direct antitumor effects by 2DG on an adenocarcinoma model (W256) known to have high glycolytic rates. We further studied the direct antitumor effects of intraperitoneal doses of 2DG on an intracerebrally implanted brain tumor model (9L gliosarcoma) with lower glycolytic rates and known radioresistance. The pharmacokinetics of FDG, adjunctive effects of FDG combined with radiation therapy and toxicity, were studied and compared with the results of the prior W256 study.

No significant differences in tumor size was noted when comparing control animals (IC tumors + sham IP injections) with either radiation (2600 rad whole brain) alone or 2DG (0.8 g/kg TID IP x 7 days) alone. Small, minimally significant differences were noted when comparing controls with the 2DG combined with radiation group. Quantitative autoradiography (QAR) of a subgroup of rats which received TID doses of 2DG showed modest increases in 2DG concentration in tumors compared to normal brain, similar to, but less than, that of the W256 model. 2DG QAR showed lower glycolytic rates in the 9L tumors which are much lower than the glycolytic rates of the W256 IC tumor model. This provides a likely explanation for the relative insensitivity of the 9L tumor to 2DG as therapy.

Therefore, pharmacologic doses of 2DG have modest direct antitumor activity, and may be useful adjunctive agents when combined with radiotherapy. Tumor cells may also be sensitized to radiation-induced sublethal DNA damage by 2DG inhibition of repair mechanisms. Tumor response can be predicted from a knowledge of the glycolytic rate compared to the normal brain, allowing selection of subjects for consideration of this therapy based on tumor glycolytic rates. 2DG clearance from tumors is slower than from normal brain, indicating 2DG will accumulate faster and persist at a higher level in tumor than normal brain. However, significant systemic and neurologic toxicities were associated with the dose regimen employed; cardiac and/or renal toxicity appeared dose limiting.

6. Immunotherapy of Brain Tumors - David Barba, M.D., Stephen Saris, M.D.

Investigation of the efficacy and toxicity of adoptive immunotherapy using interleukin-2 (IL-2) and lymphokine activated killer cells (LAK) for treatment of brain tumors included investigation of the antitumor effect of these therapies in vitro, in brain tumor models in laboratory animals, and in clinic.

a. Clinical Studies

Three patients with malignant gliomas were treated with intravenous interleukin-2 to assess the tolerance of patients with brain tumors to systemic therapy with IL-2 and LAK. All three patients developed rapid clinical progression and greatly increased cerebral edema surrounding their tumor and therapy had to be discontinued. Quantification of the degree of

cerebral edema in the surrounding brain using CT scanning and magnetic resonance imaging confirmed the clinical observation of enhanced cerebral edema induced by the treatment. This experience suggested the need to consider regional therapy if adoptive immunotherapy using IL-2 and LAK could be tolerated by patients with primary brain tumors. Two treatment protocols using regional delivery of IL-2 and LAK cells at the site of malignant primary brain tumors are now active. One protocol investigates the clinical response to repeated doses of LAK cells and IL-2 into the tumor cavity of cystic malignant brain tumors (David Barba, M.D.). Six patients were treated with this protocol. Our experience indicates that all patients develop increased brain edema in the tissue surrounding the tumor and experience some increase in focal neurological dysfunction, which is often temporary and reversed by dexamethasone treatment. Two of the six patients have had partial tumor responses, as measured by contrast enhancing CT scans, following completion of therapy. In the other clinical protocol LAK cells and IL-2 are directly injected into a solid intrinsic brain tumor (Stephen Saris, M.D.). One patient has been treated using this approach. The early post treatment CT scans suggest a partial tumor response to the treatment. However, prominent cerebral edema surrounded the brain tissue in the region of treatment and the patient had increased neurological dysfunction as a result of the treatment.

These studies suggested that there was some, although incomplete, response of the tumor to this treatment and, therefore, investigation for further development and refinement of this approach is justified. Several laboratory efforts were initiated to increase our understanding of the effect of IL-2 and LAK cells on brain tumors, and to explore the mechanism of the increased cerebral edema seen during treatments.

The antitumor effect of LAK-IL-2 therapy was investigated using electron microscopy to visualize the structural interaction between LAK cells and glioma cells during co-incubation. This work was performed by and under the supervision of Dr. Gregory Hook and is described in a separate Section of this report.

To determine whether treatment with IL-2 alone had antitumor effect in vivo, 9L glioma was implanted into the brains of rats which received therapy with systemic injection of IL-2. The survival of rats which received IL-2 was significantly increased compared to rats receiving placebo. This is described in greater detail in the segment of the report of the Neuroimmunology Unit.

To examine the mechanism of the increase in cerebral edema associated with IL-2 therapy, rats bearing 9L gliosarcoma intracerebrally were treated with IL-2; the site and degree of cerebral edema was determined using a radioactive tracer. IL-2 was shown to elicit increased cerebral edema in the zone of tissue surrounding the site of tumor implantation and to increase the accumulation of extracellular fluid within the tumor. However, the contralateral hemisphere (the normal hemisphere) was unaffected. This suggested that the enhanced cerebral edema effected by IL-2 in vivo is a result of increased vascular permeability of the vessels within the tumor and not a direct effect on the vasculature of the central nervous system. This was further investigated by incubating IL-2 with peripheral blood lymphocytes

and using the Miles assay (a bioassay of vascular permeability), to demonstrate that the co-incubation of IL-2 and peripheral blood lymphocytes results in the production of increased vascular permeability activity in the conditioned media. This demonstrates that a probable source of the increased cerebral edema surrounding brain tumors is a soluble product resulting from the interaction of IL-2 and peripheral blood lymphocytes. (This work is described in greater detail in the report of the Neuroimmunology Unit.)

A pharmacokinetic advantage of intracarotid versus intravenous administration of IL-2 for delivery to the brain was demonstrated using radiolabeled IL-2. A 10-fold pharmacokinetic advantage for brain delivery was demonstrated with intracarotid compared to intravenous infusion. Therefore, it might be possible to deliver effective doses of IL-2 to tumor bearing regions of the central nervous system at doses below the threshold for stimulation of the peripheral blood lymphocytes to produce the agent responsible for the increased vascular permeability. Similarly, it was demonstrated that a 10-fold pharmacokinetic advantage could be obtained by intracarotid infusion of LAK cells compared to intravenous administration (see Neuroimmunology Unit report).

7. Systemic Chemotherapy - Spiromustine Phase I Study - Conrad Kufta, M.D.

The disease specific phase I trial of spiromustine, a drug synthesized to cross the blood brain barrier and function as a tumoricidal agent, was closed to patient accrual in 1986. Twenty-three patients with high grade glioma and one patient with metastatic paraganglioma were entered into the study; they were treated at escalating doses until a maximum tolerated dose (MTD) was identified.

The dose-limiting toxicity of spiromustine was found to be an acute neurologic syndrome manifested chiefly as acute confusion with visual and auditory hallucinations and fixed dilated pupils. This syndrome was identified as an anticholinergic effect by the rapid reversal of its components with parenteral physostigmine. Despite antagonism of the cholinergic effects of spiromustine with physostigmine, at doses above 9.9 mg/m² a substantial number of patients became stuporous or comatose for short periods after treatment. This is considered unacceptable in patients with increased intracranial pressure. Hence, the MTD was defined at 9.9 mg/m².

Clinical response, determined within the limits of a phase I study, suggested activity. One patient has a complete remission sustained over twenty-eight months and eight additional patients had partial responses determined by clinical and/or CT scan criteria.

No permanent or late adverse effects have been noted to date on neurologic, neuropsychologic, hematologic or general physical examination. There is virtually no myelosuppression at any dose level, even in heavily pretreated patients, which suggests a potential role for spiromustine in combination chemotherapy protocols if data from phase II trials confirm these early results.

Follow-up of survivors continues for evidence of tumor progression or evidence of late-developing toxicities.

Pituitary Tumors

1. Venous Sampling to Establish the Diagnosis and Location of Hormone-Secreting Pituitary Microadenomas

We have now performed bilateral and simultaneous inferior petrosal sinus sampling in 150 patients with Cushing's syndrome. The results are used 1) to confirm the diagnosis of Cushing's disease preoperatively and, 2) to determine the half of the pituitary gland in which a microadenoma resides. The study has been particularly rewarding and has demonstrated the following: 1) sampling from both inferior petrosal sinuses simultaneously and consistently distinguishes patients with Cushing's disease from those with ectopic ACTH syndrome (greater than 99% accuracy); 2) sampling from a single inferior petrosal sinus, as has previously been general practice, to establish the diagnosis of Cushing's syndrome, may be misleading and could result in an incorrect assumption of the source of excess ACTH secretion in as many as 43% of patients with Cushing's syndrome; and 3) preoperative sampling for ACTH concentrations in the inferior petrosal sinuses determines the site of ACTH-secreting microadenomas within the pituitary gland with about 85% accuracy. Therefore, bilateral sampling permits the surgeon's search for small microadenomas to be focused to one side of the gland, which should be helpful in finding smaller tumors. If no tumor is found, the half of the gland containing the microadenoma can be removed. We have had only two treatment failures in over 60 previously untreated patients with Cushing's disease who have undergone preoperative sampling. This technique is now being widely employed in the evaluation of patients with Cushing's syndrome.

We investigated the use of additional pituitary hormones (prolactin, TSH, and alpha subunit of HCG), which are presumably secreted symmetrically into the cavernous sinuses and inferior petrosal sinuses by each half of the pituitary gland, to see if they could be used to standardize ACTH measurements against unequal dilution of the venous blood in the two inferior petrosal sinuses for tumor localization. None of these hormones proved helpful as a means of measuring unequal dilution of venous blood between the pituitary gland and the inferior petrosal sinuses. However, these hormones, particularly prolactin were consistently elevated in the blood from the inferior petrosal sinus on the same side of the tumor. This suggests that the hormonal secretion of pituitary microadenomas influences the hormonal secretion of the adjacent, normal, pituitary tissue and supports the possibility of functional endocrine regulation of hormonal secretion in the anterior lobe of the pituitary gland.

2. CT Scanning and Cushing's Syndrome

To evaluate the CT scan as a means of aiding the differential diagnosis of patients with Cushing's syndrome and as a mechanism for tumor localization

pre-operatively, we prospectively evaluated the results of CT scanning in 57 patients with Cushing's syndrome. All 8 patients with ectopic ACTH secretion had normal CT scans, all 8 patients with pituitary microadenomas had focal pituitary abnormalities that were visible on the CT scan, but only 7 of the 42 patients with pituitary microadenomas and Cushing's disease had demonstrable abnormalities on CT scanning. The conclusion of this study is that CT scanning is only occasionally helpful in locating, pre-operatively, the site of an ACTH-secreting pituitary microadenoma, and is therefore only rarely beneficial as a diagnostic aid in the differential diagnosis of patients with Cushing's syndrome.

3. Repeat Pituitary Surgery in Patients with Cushing's Disease Despite Previous Treatment

The ideal treatment of patients with Cushing's disease should eradicate tumor, reverse hypercortisolism, and avoid the requirement of longterm endocrine deficiency and permanent hormonal replacement therapy. These goals of therapy are only met with selective removal of the offending pituitary adenoma. However, patients who have received prior pituitary treatment, but still have hypercortisolism, are usually not considered candidates for repeat surgery. We investigated the role of repeat transsphenoidal surgery in 18 consecutive patients with prior pituitary treatment and persistent, or recurrent, Cushing's disease. In 10 of the 18 patients an endocrine cure was achieved by repeat transsphenoidal surgery. Our experience demonstrates that in patients with prior pituitary treatment, if an adenoma can be identified intraoperatively and selectively excised, the patient can be expected to have resolution of hypercortisolism and preservation of normal pituitary function. They further indicate that if a distinct adenoma cannot be identified intraoperatively, the chances of effecting resolution of hypercortisolism and preserving pituitary function are quite low.

Surgical Treatment of Medically Intractable Epilepsy

Conrad Kufta, M.D.

The aim of the surgical arm of the NINCDS epilepsy program is to develop surgical methods for more precise localization and safer resection of epileptogenic foci. The development and implementation of surgical treatments for epileptic patients intractable to all other medical and surgical therapies is the immediate and long-term goal of this program.

Several surgical procedures and methodologies have been introduced during the past 2 years in the ongoing attempt to better select and treat patients with medically intractable epilepsy. Specially designed and targeted sphenoidal electrodes are now being implanted to help localize epileptogenic foci which originate in the medial temporal lobe and from which electrical projection to the lateral temporal surface can lead to false localization of epileptogenic source by surface EEG.

Subdural surface electrodes which were designed and built at NIH in collaboration with the BEI branch of DRS are being implanted in selected patients to record directly from the cortical surface for longer periods than is possible with electrocorticography. These electrodes are also being utilized for direct repeated stimulation of cortical foci to better discriminate motor, sensory, and language areas. Such discrimination helps in the topographical identification of overlap between critical cortical areas and epileptic foci during surgical procedures.

During surgery for focal epilepsy, depth and special subdural (surface) electrodes are used to record from deep structures inaccessible by routine electrocorticography to identify and confirm areas of potential epileptogenic activity suggested and localized by the new preoperative diagnostic methods of PET scanning, MRI and magnetoencephalography (MEG).

MEG offers in theory the possibility for localization of abnormal seizure-related electromagnetic phenomena in 3-dimensions. No other methodology currently available can noninvasively acquire this type of information which is highly desirable for identifying epileptogenic foci for surgical excision and in the study of basic mechanisms in epilepsy. In one notable case to date, using MEG data we were able to predict correctly the localization of a focus causing complex, partial seizures in a patient whose preoperative EEG (in retrospect) gave false localizing information. This focus was successfully extirpated and would have been missed if a standard temporal lobectomy had been performed. A new 7 channel MEG, now in place, promises even greater 3-dimensional localizing capability, and we are now studying focal motor seizures as well as complex partial seizures with this technique.

Electrocorticography at the time of resective surgery often generates data of uncertain significance. A study initiated during the past year is carefully evaluating the effect of different anesthetic agents on interictal spikes, background activity, and the relationship between abnormal and normal background activity. Information derived from this study should better define and delineate activity which is associated with true epileptogenic foci and hence enable more accurate and smaller focal resections to be accomplished.

As abnormal foci which appear to cause clinical seizures are better defined an attempt will be made to identify chemical derangements in these focal areas. One approach includes the measurement of neuropeptides and transmitter substances in fresh, frozen tissue samples of epileptogenic cortex, removed during resection surgery. This work is being done in collaboration with Dr. Suzanne Nadi, MNB. A second approach will utilize NMR spectroscopy for in vivo focal pH measurements, and the measurement of phosphorus containing compounds, especially those involved in energy transfer. As predicted technical developments advance, we plan to measure focal extracellular water content during early and late postictal periods when sodium spectra can be obtained and quantified. This work is in collaboration with Dr. Jeffrey Alger and Dr. Giovanni Di Chiro of the Neuroimaging Section.

One of the most severe postoperative deficits suffered by patients with temporal lobectomy is unpredictable recent memory loss. It is assumed that this is due to removal of hippocampus in patients who have poor hippocampal function on the side opposite the resection. Routine Amytal (WADA) testing has been only minimally successful in predicting post-op memory deficits in patients considered for unilateral hippocampectomy for seizures. Therefore over the next 2 years a study of (super selective) posterior cerebral artery Amytal testing will be done in collaboration with Dr. John Doppman of the Radiology Department, CC, which will attempt to selectively block hippocampal function unilaterally in the awake patient leading to predictions about the state of postoperative memory function after this tissue is removed.

Vascular Disorders of the CNS

1. Spinal Arteriovenous Malformations

A retrospective review of the clinical features, arteriographic findings, and treatment of 81 patients with spinal arteriovenous malformations demonstrated that there are distinguishing clinical features in patients with arteriovenous malformations of the spinal cord compared to those of patients with arteriovenous fistulas of the spinal dura. They also suggested that the demographic characteristics of spinal arteriovenous fistulas suggest that this is an acquired and not congenital lesion and support true spinal arteriovenous malformations of the spinal cord as congenital lesions. The findings indicate that true arteriovenous malformations of the spinal cord produce myelopathy as a result of the high flow through these lesions with its associated arterial steal phenomenon and the multiple hemorrhages experienced by these patients with spinal dural AV fistulas develop myelopathy as a result of increased pressure of the venous system of the spinal cord. The results also demonstrate that the response to treatment is better with patients with spinal dural AV fistulas and, if the patient's fistula can be identified early in the course of the illness, that most neurological function of the spinal cord can be preserved by treatment.

2. Magnetic Resonance Imaging in Patients with Spinal Arteriovenous Malformations

Magnetic resonance imaging, used prospectively to evaluate patients with spinal arteriovenous malformations, demonstrated the presence and the site of arteriovenous malformations of the spinal cord. However, it failed to demonstrate the site of, and only occasionally demonstrated the presence of, spinal dural AV fistulas. In patients with arteriovenous malformations of the spinal cord it permitted sequential assessment of the success of therapy to obliterate the malformations. Therefore, it proved to be a valuable and noninvasive technique to investigate patients suspected of having spinal arteriovenous malformations and to sequentially follow the course of these lesions during and after therapy.

3. Cerebral Arteriovenous Malformations

Although advances in the measurement of cerebral blood flow (CBF) have been achieved in recent years, techniques that permit continuous rapid determination of CBF are limited to thermal-dilution and hydrogen-clearance methods and to a more recently developed technique, laser Doppler velocimetry. This technique was used to measure microcirculatory flow continuously in a patient undergoing surgery to excise a cerebral arteriovenous malformation. The physiological changes associated with excision of a cerebral arteriovenous malformation were successfully examined using CBF recorded intraoperatively with the laser Doppler probe. These findings indicate impaired vasoregulation in the normal brain surrounding the AVM and support the perfusion pressure break through theory as responsible for the perioperative brain swelling in these patients.

4. Entorhinal (perforant pathway) Ablation Studies in the Rat - Don Wright, M.D.

Brain injury (ischemia/infarction, hemorrhage) by atherosclerotic cerebrovascular disease constitutes a clinically important entity resisting adequate treatment once the injury has become apparent. The excitatory (glutamatergic) innervation of several brain regions appears to determine the neuronal vulnerability to complete transient ischemia. Interruption of the excitatory afferents to such regions (hippocampus/dentate) prior to ischemia allows examination of this hypothesis. A series of experiments have been performed to examine the functional anatomic connections of regions known to manifest selective vulnerability to ischemia and the influence on metabolic rates of glucose consumption following complete transient ischemia.

Functional/anatomic connections of the dentate/hippocampal complex were examined in Wistar rats by 2DG QAR. Unilateral and bilateral entorhinal ablative lesions induced regional changes in metabolic function as evidence by $LCMR_{glu}$ in rats within hours following such lesions, and by comparing the changes in metabolic activity with analogous histologic sections of brain, we deduced functional connections of this region.

Our findings support the hypothesis that the perforant pathway (entorhinal afferents) have a functionally significant terminal field in the stratum lacunosum-molecular of the CA1 neurones. The protective effect of lesions of this pathway on the CA1 neurons against ischemic neuronal death appear to be mediated through field.

5. Selective Vulnerability/Ischemia Studies in Rats - Don Wright, M.D.

It is proposed (excitotoxic theory) that the selective vulnerability of neuronal populations is partly mediated by excitatory afferents, and the relative hypermetabolic response to ischemia injury can be modified by various stimulation or ablative maneuvers. A group of normal Wistar rats were subjected to total ischemia (4 vessel occlusion-hypotension model) following

ablative procedures (Kainic acid injections, or entorhinal ablations). The LCMR_{glu} was then examined with QAR to assess the metabolic pattern in the dentate/hippocampal regions and the results were correlated with analogous histologic sections as well as control rats.

The hypermetabolic patterns in specific brain regions subject to excitatory afferent input were readily influenced (abolished, decreased, etc) by altering the afferent input to the CA1 and CA3 regions of the dentate gyrus. Functional and anatomic connections also can be determined and their relative roles in selective neuronal vulnerability to ischemia can be established by examining the metabolic patterns after such ischemic injury. Prior methods have not successfully employed functional (metabolic) mapping using QAR. We are currently investigating means of modifying the selective vulnerability of dentate/hippocampal neurons to ischemic insults.

B. BIOCHEMISTRY UNIT

Richard J. Youle, Ph.D. - Head

1. Monoclonal Antibody Mediated Killing of Tumor Cells

The Unit of Biochemistry, headed by Dr. Richard Youle, is studying the use of monoclonal antibodies to kill disease-causing cells. Monoclonal antibodies which selectively bind tumor cells can be generated, but alone are usually not cytotoxic to the tumor. Toxic proteins such as ricin and diphtheria toxin can be chemically linked to monoclonal antibodies. The new hybrid molecules bind tumor cells via the antibody moiety and then kill the cells via the toxin moiety. The toxins used are enzymes that catalytically inactivate protein synthesis in target cells with only one or two molecules in the cytoplasm killing a cell. However, the non-target cell toxicity of the toxins must be blocked with excess lactose to prevent toxin binding and this currently limits this approach to in vitro applications. The cell-type-specific reagents have immediate clinical application in vitro in bone marrow transplantation where T cell depletion improves allogeneic transplantation. The Unit of Biochemistry is collaborating in clinical trials in bone marrow transplantation at the University of Minnesota. Twenty-four patients have now been treated with immunotoxin purged bone marrow as the sole prophylaxis for graft-versus-host disease. Comparing the outcomes with historic controls treated post-transplant with methotrexate, several conclusions can be drawn. The patients had a milder course as evidenced by a significantly shorter hospitalization. Engraftment of donor marrow occurred with a shorter time until leucocyte generation and no severe graft-versus-host disease was seen. Clinical trials of immunotoxin treatment of bone marrow are continuing to increase the patient population and thus the statistical significance of the apparent benefits.

The major goal of the laboratory is to develop immunotoxins which will selectively kill tumor cells in vivo. Currently the limiting steps for antibody-toxin hybrids are (1) the entry of the toxin molecule into the cell; and (2) in vivo access of the drug to the tumor cells.

Several new approaches have been successfully applied in vitro and in vivo in the past year to overcome these limitations.

To promote access of monoclonal antibody-toxin conjugates to tumors we have focused on tumors localized in body compartments such as the brain and the peritoneal cavity. The brain may be an optimal compartment for antibody modulation of cell function.

- a. We developed 2 animal models of carcinomatosis meningitis. 1) Human leukemia cells, CEM, are injected intracerebrally or into the CSF via the cisternum magnum, into nude rats. Ten to fourteen days later the animals die due to the brain tumor; and 2) guinea pig leukemia cells, L2C, were injected into the CSF of guinea pigs resulting in death 15 days later.

- b. We have localized a monoclonal antibody to the solid tumor in the cerebral tumor model. The surprising and encouraging result is that the antibody not only bound to CSF borne cells but also bound cells several millimeters into the brain parenchyma.
- c. We have synthesized an antibody-toxin conjugate that binds the transferrin receptor and binds human brain tumor cells 5 times more than normal human brain cells. In vitro the antibody-ricin conjugate specifically kills tumor cells via the tumor-associated antigen.
- d. We have treated a peritoneal tumor with peritoneally injected antibody-toxin conjugate and achieved 12% long-term survivors and an extension in mean survival time indicating a mean 5-log kill of tumor cells in vivo. We are currently considering adapting this procedure to clinical testing in humans with ovarian cancer.
- e. We have treated CSF born tumor with CSF injected antibody-toxin conjugate and achieved 8% long-term survivors and an extension in mean survival time showing a mean 2 to 3-log and in some animals a 5-log kill of tumor cells in the brain.

To improve entry of immunotoxins into cells we have modified toxins chemically, molecular biologically and with monoclonal antibodies. We are studying the structure-function relations of the toxin molecule and the cell biology of toxin internalization and membrane penetration.

- a. We raised a panel of monoclonal antibodies (MoAb) to study the various activities of ricin. One antibody binds the lactose binding site on ricin and affects immunotoxins in vitro like lactose. In vivo the MoAb protects mice from ricin toxicity like asialofetuin. Monoclonal antibodies stay in circulation for days and should be even better than asialofetuin for blockage of ricin B chain. We are initiating in vivo therapy of cancer in animal models using intact ricin immunotoxins plus the anti B chain monoclonal antibody against the ricin binding site.
- b. Toxins may be best adapted for tumor specific toxicity by alterations of amino acid sequence at the gene level. To begin improving immunotoxins at the gene level we have worked with the prokaryotic toxin, diphtheria toxin. Intact diphtheria toxin was linked to a monoclonal antibody specific for human T cells and was found to specifically kill target cells at $10^{-12}M$. The rate of specific killing was 10-fold faster than previously reported immunotoxins. This model system was then used to study cloned fragments of diphtheria toxin. In collaboration with Dr. Larry Greenfield, who has cloned diphtheria toxin (DT), we deleted the C-terminal 15KD region shown to include the cell surface binding site. This left the toxin A subunit plus a 17 KD fragment of DT B chain thought to facilitate transmembrane transport. When linked to monoclonal antibodies, this truncated DT was 100-fold more toxic than DTA chain linked to antibody and the toxicity was blocked by excess antibody. The cloned DT fragment was 1000 times less toxic to guinea pigs than the native toxin. Therefore, the fragment of DT B chain included by cloning

increased target cell toxicity more than non-target cell toxicity indicating that separation of B chain entry functions from binding was accomplished to some degree. We compared intact DT linked to monoclonal antibody with the cloned fragment of DT, which showed the C-terminal fragment of DT further increased the antibody mediated toxicity 100-fold. We are currently studying conjugates of 3 point mutants of diphtheria toxin linked to antibodies in attempts to include the 100-fold activation by the C terminus of DT while omitting regions causing non-target cell killing. Recent results show that point mutations can increase selective cytotoxicity up to 10,000-fold.

- c. We are studying the mechanism of toxin entry into cells to allow improved design of tumor-specific reagents. Toxins like ricin and diphtheria toxin bind the cell surface, are endocytosed, then cross the membrane surrounding the endocytotic vesicle to reach the cytosol where they inactivate protein synthesis. The rate limiting step in toxin and immunotoxin activity is transport across the membrane to the cytosol. How and where this transport step occurs is unknown. To investigate this question we have used hybridoma cells which secrete monoclonal antibodies that block ricin toxicity. We have found that these cells are themselves resistant to ricin because of the antibody they synthesize. We found that the resistance was not caused by extracellular or cell surface antibody but by antibody within the cell in route to secretion. This means that ricin must pass through the protein secretory pathway, comprising the endoplasmic reticulum, golgi apparatus, and secretory vesicles, before entering the cytosol. This new approach may be applied to study other toxins, hormones and macromolecules which enter cells by receptor mediated endocytosis.

2. Programmed Cell Death in the Nervous and Immune System

Our laboratory has begun a project to study the mechanism and physiologic role of programmed cell death. In both the nervous system and the immune system, massive numbers of cells die during normal development. In the spinal cord, for example, over half the neurons die during fetal development. The immune system also has large cell loss at precise stages in development such as thymocyte death and thymus involution at puberty. The physiologic role and the biochemical mechanism of programmed cell deaths is unknown. Understanding the mechanism and regulation of such physiological cell deaths may shed light on neurodegenerative diseases and immunodeficiency disorders. One rare hereditary disease, Ataxia telangiectasia, demonstrates the relationship between cell death in the nervous and immune systems. This disease results from a single recessive gene and is characterized by Purkinje cell death and premature thymus involution. Patients are ataxic but usually die from infections resulting from a pronounced immunodeficiency.

In our initial study we found that thymocytes could be induced to die by the same signal that stimulates mature T cells to proliferate. RNA and protein synthesis inhibitors block new gene expression and block the programmed cell death. This is consistent with a new model of immune system

self-tolerance that states that potentially auto-reactive cells are induced to die by cross-linking the T cell receptor early in development, the same signal that induces mature T cells to react against foreign antigens.

We are extending our studies into the nervous system by examining the mechanism of thymocyte death in various ataxic mutant mice and by examining the mechanism of glutamate induced neuron death in vitro and in vivo. We are also studying the effect of pituitary hormones on programmed cell death.

C. Central Nervous System Implantation Unit

Robert J. Plunkett, M.D., Head

1. Development of Primate Model of Unilateral Parkinson's Disease

We have developed a new model of Parkinson's disease which allows *in vivo* quantification of response to therapeutic maneuvers. Systemic administration of MPTP selectively injures the nigrostriatal pathway in primates. Affected monkeys provide a valuable model of Parkinson's disease. Since after IV MPTP the basal ganglia are affected bilaterally, there is no unaffected striatum in the same animal to serve as control tissue for comparison, and the neurological deficit is so severe that the animals require L-DOPA administration or intensive care to survive. The extremely short plasma half-life of MPTP suggested to us that infusion of MPTP into the internal carotid artery (ICA) would cause unilateral Parkinsonism.

Graded doses of MPTP, 0.2-1.2 mg/kg, were infused into the ICA of adult rhesus monkeys. Animals that received 0.4 to 0.8 mg/kg developed rigidity, bradykinesia, posturing deformity, involuntary movements, and resting tremor of the contralateral extremities and rotation toward the infused side. The extremities ipsilateral to the infusion were unaffected, which permitted the animals to move about freely and feed themselves without difficulty. Treatment with L-DOPA reversed all extrapyramidal signs. Infusion of L-DOPA and apomorphine reversed the direction of rotation. The brains of animals were analyzed for morphological, immunohistochemical and chemical changes. There was an extensive loss of dopaminergic neurons in nuclei A8, A9, and A10 of the substantia nigra and prominent loss of dopamine-containing nerve fibers in the basal ganglia on the infused side, whereas the corresponding tissues in the opposite half of the brain appeared normal. The dopamine and homovanillic acid content in the perfused putamen and caudate nuclei were less than 4% of those in the contralateral hemisphere.

Intracarotid injection of MPTP causes degeneration of the nigrostriatal pathway only on the side of infusion. The rate of rotation of affected animals can be quantified and used to assess the response to therapy. This primate model of Parkinson's disease should be useful for further investigation of Parkinson's disease and of the responses of normal, contralateral brain to unilateral nigrostriatal injury. It should also prove useful in the investigation of tissue implants in Parkinson's disease in primates.

The behavioral, biochemical, and histological affects of tissues implanted into the brain of mammals is being studied. Rodent and primate models of Parkinson's disease are recipients for dopaminergic tissue implants. Fetal mesencephalic grafts to the striatum have been successful for restoration of function in the primates. We have also observed sprouting from MPTP undamaged dopaminergic cells in the implanted animals. This may be the basis of the functional recovery in these animals and is now under active investigation.

2. Dopaminergic Implants in Parkinsonian Primates

Fetal substantia nigra tissue grafts were placed into previously created cavities in the caudate nucleus of MPTP-induced Parkinsonian primates. A similar procedure was used to graft adrenal medullary allografts. Both groups of animals demonstrated a significant functional recovery. However the recovery in animals which received the adrenal medullary allografts was transient and no surviving tissue could be demonstrated by immunohistochemistry at six months. In contrast, there was a prominent degree of functional recovery in the animals which received fetal midbrain implants that persisted and immunohistochemical techniques demonstrated survival of the grafts in these animals. In addition, abundant sprouting of dopaminergic fibers from the host brain toward the implant cavity occurred in these animals. A qualitatively similar sprouting of the host brain was demonstrated in animals which received the adrenal medullary grafts, although it was somewhat less prominent.

3. Stereotactic Implantation of Dispersed Cells in the Brain

This systematic study was performed to perfect the technique for implantation of dispersed cells into the brains of mammals. The effect of cell concentration, rate of infusion, volume, and needle size on the success of placement of the dispersed cells in the central nervous system of rats and of primates was investigated. An accurate, reproducible method of cell placement into the caudate nucleus of rats and primates was demonstrated. Eighty percent of the radioactivity was present at the target site, and at least half the radioactivity was within viable cells using injection volumes of 2.5 to 10 microliters, 26 gauge Hamilton syringes, an infusion rate of 1 to 5 microliters per minute and a cell concentration from 2×10^6 to 2×10^8 cells per ml.

Part of our effort has been to perfect the technical aspects of stereotactic implantation of dispersed cells into brain. We have identified the factors which influence the accuracy of cell placement, and will now use this information to implant various dopaminergic cells into the brains of lesioned animals.

D. Tumor Biology Unit

Marsha J. Merrill, Ph.D., Head

1. Vascular Permeability Factor Produced by Glioma Cells

We have determined that medium conditioned by cultured human glioma cells contains a substance capable of increasing vascular permeability. This vascular permeability factor (VPF) is probably at least partially responsible for the cerebral edema associated with brain tumors. VPF appears to be a heparin-binding protein which is distinct from other substances known to elicit edema or regulate endothelial cell growth. We are now in the process of purifying VPF so that we can characterize the molecule, the regulation of its production by the tumor cell, and the mechanism of action of VPF on the cerebral vasculature. As purification progresses we will attempt to raise a monoclonal antibody to VPF. This may provide a means of obtaining further insight into the basic mechanisms of brain tumor associated edema.

2. The Role of Insulin and Insulin-Like Growth Factors in Glioma Cells

Insulin and insulin-like growth factor (IGF) receptors have been detected in the human central nervous system. The role of IGFs in the nervous system is not well understood although IGF-I may be involved in the growth of the peripheral nervous system and IGF-II may play a role in glial cell growth and maturation in the brain. The role of insulin and IGFs in glioma cells has not yet been investigated. We have initiated studies to determine the nature of insulin and IGF receptors on human glioma cells derived from surgical specimens. Our results demonstrate 1) high levels of IGF-I receptors on low grade astrocytomas; 2) the presence of receptors for IGF-I and IGF-II but not for insulin on cultured cells derived from human gliomas; and 3) the presence of an IGF-like substance in serum-free medium conditioned by cultured glioma cells. Our findings suggest a role for IGF's in glioma cell biology. The functional significance of IGF receptors is being assessed by correlating the receptor binding of IGFs with the effects of IGFs on cell metabolism. These cultured cells may also provide a model system for study of the effects of IGFs on glial cells.

E. NEUROIMMUNOLOGY UNIT

Richard J. Weber, Ph.D., Head
David Barba, M.D. and Stephen Saris, M.D.

The Neuroimmunology Unit is dedicated to the study of immunotherapy of brain tumors as well as study of the related issue of immunosuppression found in subjects with malignant glioma. We have used a tumor model system involving the intracerebral injection of autologous gliosarcoma cell line in rats followed by a systematic evaluation of the efficacy of interleukin-2 (IL-2) and lymphokine activated killer cells (LAK) in the treatment of such tumor bearing animals. This model system allows the evaluation of such parameters as route of administration of IL-2 or LAK, dosage, optimization of in vitro tumor cell killing and immunohistochemical and autoradiographic analysis of leukocytes which infiltrate glial tumors.

Initial studies demonstrated that IL-2 administration alone was successful in extending the survival time of tumor bearing rats. IL-2 was administered to tumor bearing animals for 5 days TID while control animals received equal injections of the vehicle. Animals treated with IL-2 showed a 20% increase in survival compared to the control animals, 25.4 $\pm$ 3.8 days versus 21.4 $\pm$ 2.7 days after implantation respectively ($p = 0.002$). Preliminary evidence suggests that IL-2 induces leukocytes to infiltrate the brain tumor and offers a possible explanation of this phenomenon. Fluorescent immunoassays, both fluorescent activated cell sorter analysis (FACS) and immunohistochemical analysis of rat leukocyte cell surface, have been worked out in preparation to identify the cell types which may be responsible for the observed IL-2 effect. It was also shown that injection of IL-2 via internal carotid artery results in a 20-fold increase in brain delivery versus venous administration.

Culture of density gradient-separated leukocytes in recombinant IL-2 results in the differentiation of rat splenic leukocytes into tumoricidal LAK. We optimized the conditions for generation of LAK with respect to IL-2 dosage and time course and parameters for storage of LAK under liquid nitrogen in preparation for use. Robert Plunkett has shown that intracranial administration of chromium-51 labelled LAK cells was optimal at 2×10^7 cells/ml in 10 microliters at 5 microliter per minute via 23 gauge Hamilton syringe needles. Recovery of lymphocyte associated radioactivity from brain after dissociation was nearly 50 percent (see CNS Implantation Unit). These results have established the optimal parameters and conditions to allow a rigorous evaluation of IL-2 and LAK immunotherapy in the treatment of malignant glioma. These results are expected to guide the clinical staff in implementing the various clinical protocols underway designed to treat malignant glioma in patients.

A severe limitation of IL-2 immunotherapy stems from the toxic side effects of IL-2 therapy resulting in a vascular leak syndrome. We showed that IL-2 induces the in vitro production of a leukocyte derived vascular permeability factor (L-VPF) from human peripheral blood monocytes. Preliminary characterization of L-VPF has demonstrated its micromolecular

character (greater than 30 Kd). We are currently attempting to identify the cellular source of L-VPF and further elucidate the molecular character of this factor. Knowledge in this area may allow us to design methods to eliminate L-VPF production without sacrificing the immunopotentiating effects of IL-2.

Previous work by us and others has shown that stressors, both physical and psychological, can adversely affect immune responsiveness. We have demonstrated that conditioned fear results in a rapid and dramatic suppression of immune function. We have shown that patients with panic disorder when undergoing panic attack exhibit alterations in responsiveness of peripheral blood lymphocytes, both cytotoxic and mitogen induced.

We are studying the mechanisms of neural control of immunity by using drugs which affect immune functions and which act in the central nervous system. Since stress exposure can lead to the release of opiate peptides centrally and peripherally, we have studied the involvement of opiates in immune regulation. Direct in vitro effects of opiates on immune function coupled with the identification of opiate receptors on leukocytes, and production of opiate peptides by these cells have led us to speculate that the opiate system functions as an internal regulator of the immune system and, therefore, opiate peptides fulfill the criteria for being classified as interleukins. Indeed, B-endorphin can activate a specific population of lymphocytes into cytotoxic cells against tumors which are normally natural killer cell resistant.

Effects of opiates in vivo suggest that opiates act indirectly to modulate immune function. We have shown that chronic administration of morphine suppresses T cell functions involved in antibody production in rats and mice. Furthermore, it has been established that cytotoxic responses can be affected by in vivo opiate administration. We have extended this work to include the LAK cell phenomenon.

In order to localize the CNS effects of morphine on immune function, rats were implanted with bilateral cannulae guides aimed for various brain structures. One week following surgery the animals received bilateral injections of morphine (5 ug in 1 ul saline) in each structure. Three hours following administration of morphine the rats were sacrificed, spleens removed, and both NK cell activity and mitogen stimulated lymphocyte proliferation were measured. Injections of morphine into the anterior hypothalamus, arcuate nucleus, medial amygdala, medial thalamus, and dorsal hippocampus had no significant effect on the parameters of immunocompetence examined, when compared to uninjected controls. Injection of morphine into the periaqueductal gray matter PAG, however, produced a significant suppression of NK cell activity and T cell proliferation in response to mitogen, when compared to saline injected animals. These findings suggest that the central actions of opiates on immune function are mediated through the PAG. The precise neural mechanisms involved, however, remain to be elucidated.

F. ELECTRON MICROSCOPY UNIT

Gregory Hook, Ph.D, Head - Departed the NIH on June 1987

1. Morphology of LAK-Glioma Cell Conjugates

Culture of peripheral blood mononuclear cells (PBM) with recombinant interleukin-2 (rIL-2) results in lymphokine activated killer cells (LAK) that can kill fresh non-cultured, natural killer cell resistant tumor cells. Clinically, human LAK and rIL-2 immunotherapy can cause regression of some tumors in humans and trials using LAK cells to treat glioma patients are underway. LAK effector cells are a subset of the LAK population that must physically contact target cells in order to kill them. Very little is known on the cellular mechanism of LAK cell killing of target cells.

We studied the structure of human LAK cells killing glioma-derived cells in vitro by scanning and transmission electron microscopy of fixed cells and high-resolution Nomarski video light microscopy of live cells. PBM cells were obtained from normal adult volunteers and stimulated in culture for 4 days with rIL-2 (Cetus, Emeryville, CA). Glioma-derived cells were obtained at surgery from a recurrent glioma patient and established as a cell line (SNB-92). SNB-92 cells were positive for glial fibrillary acidic protein and were used at passage 8. LAK activity was measured by short-term chromium-51 release assays and correlated with LAK-glioma cell conjugate morphology.

We reported the first morphological study of LAK-tumor cell conjugates. We found a subset of cells within the LAK population that underwent a cytoplasmic rearrangement upon binding to target cells which is characteristic of cytotoxic T cells and natural killer cells. These LAK-glioma cell conjugates formed very tight plasma membrane bonds with numerous interdigitations and in the small extracellular spaces between the cells were membrane fragments and vesicles. These LAK cells had an asymmetry to their subcellular architecture; the nucleus was positioned away from the target cell whereas the granules, vesicles, Golgi apparatus and microtubules were oriented towards the target cell. We never saw this morphology in unstimulated peripheral blood mononuclear cells co-cultured with tumor-target cells. The glioma-derived cells are normally flat and attached to the substrate and reacted to LAK attack by forming plasma membrane blebs, becoming round and rapidly detaching from the substrate. The injured glioma-derived cells had a highly condensed cytoplasm and chromatin and a lobular shaped nucleus but did not exhibit cytoplasmic or nuclear swelling and plasma membrane disintegration. The appearance of the dying glioma-derived cells resembled an apoptotic mechanism of cell death rather than osmotic lysis. This study supports a common cytotoxic mechanism for cytotoxic T cells, natural killer cells and LAK cells. The cytotoxic mechanism is based on the local exocytosis of granules or vesicles by the effector cell into the small extracellular space between the effector-target cell conjugate. The granules contain a pore-forming protein which inserts a hole in the target cell membrane that may allow a toxic substance to enter the target cell and cause its death.

2. Quantitative Morphometrics

The number, length, perimeter and area measurements of cells are fundamental for quantitating and statistically describing and comparing cellular structures. A computer is essential to make these measurements but our laboratory did not have a system that could make the measurements.

We developed a new software package called MacMeasure for counting and measuring structures in photographs by use of a Macintosh computer. The number, length, perimeter and area of structures can be easily measured, sequentially numbered, scaled, mapped and stored on floppy disk. The hardware for the system is inexpensive and the software is very intuitive and easy to use. The measurements can be made from any paper hard copy (photographs, maps etc.) up to 12x12 inches in size. The photo is placed on a digit pad and structures are measured by the investigator tracing the outline with a mouse. Objects are counted by the operator placing the mouse over object and pressing a button on the mouse. Lines and areas can be measured with a precision of 0.1 mm and 0.01 mm², respectively. The files of MacMeasure are completely compatible with many other commercially available statistics and graphics software packages for the Macintosh. When combined with these other software packages, measurements can be rapidly analyzed, graphed, displayed and compared. Further, if the Macintosh is connected to a Laser Writer, high quality hard copy of the display can be rapidly made for publications or slides. The MacMeasure software allowed us to quantitate structures in electron and light micrographs of cells.

We applied the system to analyze cellular structures observed in melanotrophs of the intermediate lobe of the rat pituitary. Melanotrophs are heterogeneous cells composed of "dark" and "light" cells as seen in light and electron microscopy sections. Melanotrophs secrete pro-opiomelanocortin (POMC) and in situ hybridization studies have shown the dark cells have a high POMC biosynthetic activity and light cells have low POMC biosynthetic activity. We quantitated various subcellular structures in dark and light cells and found a statistically higher concentration of endoplasmic reticulum and mitochondria in dark cells. Our dimensional analysis correlated the structure of these metabolically active organelles with the functional POMC biosynthetic activity of dark melanotrophs.

In addition, we have shown that this program can be used to measure volumes of tissue (tumor, CSF, organ size, etc.) from computed tomography and magnetic resonance imaging hard copies.

The MacMeasure software program was developed in collaboration with Mr. Wayne Rasband of the Mental Health Intramural Research Program, NIMH. The quantitation of the melanotrophs was in collaboration with Dr. Bibie Chronwall of the Experimental Therapeutics Branch, NINCDS.

G. CELLULAR BIOLOGY UNIT

Joseph Bressler, Ph.D., Head - Departed SNB on February 1987

The studies described are concerned with regulatory mechanisms important in sustaining a differentiated state in gliomas. This work has led us to try to understand the role of cAMP during differentiation and how the levels of cAMP are regulated in gliomas. These studies should eventually lead to the development of pharmacological agents which will inhibit glioma cell growth and induce differentiation.

1. Role of cAMP during glial differentiation

An hypothesis in our laboratory has been whether gliomas can be inhibited from proliferating and induced to differentiate by raising cAMP levels. This hypothesis is based on our data which suggested to us that agents which inhibit glial cells from expressing differentiated properties also decrease cAMP levels. One class of agents, which activate the protein kinase C and inhibit the expression of differentiated properties in gliomas, stimulates phosphodiesterase degradation. At least 30 minutes are needed to see the increase in phosphodiesterase activity which suggested that calcium and or calmodulin was not involved. Therefore it seems that in glioma cells a species of phosphodiesterase serves as a substrate for protein kinase C.

2. Identification of Substrates for Protein Kinase C in Glial Cells

In parallel studies to the above we found six molecular weight classes of proteins, 90K, 80K, 45K, 35K, 30K, 25K, which were consistently found to have higher levels of phosphorylation after protein kinase C was activated. In primary astroglial cultures we found four molecular weight classes of proteins, 90K, 80K, 45K, and 25K, which were consistently found to have a higher levels of phosphorylation after protein kinase C was activated. The 90K and 80K proteins were localized to the plasma membrane. Both proteins were consistently phosphorylated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02697-03 SN
PERIOD COVERED <u>October 1, 1986 through September 31, 1987</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Evaluation of Metabolic Differential Between Brain & Brain Tumor with Thiopental</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) Edward H. Oldfield, M.D. Chief, SNB, Principal Investigator, NINCDS Robert Miletich, M.D. Medical Staff Fellow, CC		
COOPERATING UNITS (if any) Giovanni DiChiro, M.D., NIS, ODIR, NINCDS		
LAB/BRANCH <u>Surgical Neurology Branch</u>		
SECTION <u>Clinical Neurosurgery Section</u>		
INSTITUTE AND LOCATION <u>NINCDS, National Institutes of Health, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <u>0.3</u>	PROFESSIONAL: <u>0.3</u>	OTHER: <u>0.0</u>
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project involves FDG-PET scanning of patients who have brain tumors before and during deep barbiturate general anesthesia. The results indicate that a profound reduction of cerebral glycolytic activity can be achieved with a level of anesthesia which produces burst-suppression EEG activity. Gliomas, however, have only a minimal change in glycolytic rate under the barbiturate anesthesia. The results also indicate that lower grade lesions, which are not visible on PET scans performed with the patient awake, become visible as background synaptic activity is suppressed with barbiturates. The true extent of growth into the surrounding tissue by higher grade lesions can be better appreciated when background activity is reduced under the barbiturate anesthesia. Since coma producing doses of barbiturates would be inconvenient for clinical use, we investigated the possibility of achieving a portion of this effect using sedative doses of pentobarbital. This resulted in the demonstration that about 50% of the suppression of glucose metabolism by normal brain using coma-producing doses of barbiturates can be achieved by pre-anesthetic (sedative) doses of pentobarbital. Therefore, using doses of pentobarbital that could be used repeatedly and conveniently in the clinic, we have achieved suppression of brain metabolism while leaving tumor metabolism unaffected. This work provides evidence that barbiturates may allow a "reverse contrast enhancement" of lesions with decreased neuronal activity. This phenomenon may provide a basis for development of specific antitumor therapy. The technique also may be valuable in studying other pathological processes such as degenerative diseases, epilepsy, movement disorders, cerebral infarction and head injury.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02739-01 SN

PERIOD COVERED

October 1, 1986 through September 31, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Laboratory Investigation of Central Nervous System Vascular Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Edward H. Oldfield, M.D. Chief, SNB, Principal Investigator, NINCDS

Donald Wright, M.D. Medical Officer, SNB, NINCDS

Martin Jorgensen, M.D. Visiting Fellow, SNB, NINCDS

COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC

LAB/BRANCH

Surgical Neurology Branch

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical features, arteriographic findings, and treatment of 81 patients with spinal arteriovenous malformations demonstrated that there are distinguishing clinical features in patients with arteriovenous malformations of the spinal cord compared to those of patients with arteriovenous fistulas of the spinal dura. The findings indicate that spinal arteriovenous fistulas are acquired lesions, and not congenital, as was previously thought, and support arteriovenous malformations of the spinal cord as congenital lesions. The findings also indicate that AVMs of the spinal cord produce myelopathy as a result of high flow and that the associated arterial steal and the multiple hemorrhages experienced by these patients result from the rapid flow through the lesions, whereas patients with spinal dural AV fistulas develop myelopathy as a result of increased venous pressure in the spinal cord.

Magnetic resonance imaging permits demonstration of the presence and site of arteriovenous malformations of the spinal cord and therefore is a valuable and safe, non-invasive technique to investigate patients suspected of having spinal AVMs.

Laser Doppler velocimetry can be used to monitor microcirculatory flow in the brain intraoperatively in patients with cerebral arteriovenous malformations. The results from using this procedure in one such patient demonstrate that the excision of the AVM leads to a prominent increase in cerebral blood flow in the tissue adjacent to the AVM and support the presence of a transient loss of brain vasoregulation in this tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02673-03 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Monoclonal Antibodies Linked to Ricin for Use in Human Bone Marrow Transplantation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard J. Youle, Ph.D. Principal Investigator, SNB, NINCDS Pat Johnson Bio Laboratory Technician, SNB, NINCDS		
COOPERATING UNITS (if any) University of Minnesota, Department of Laboratory Medicine National Cancer Institute, Immunology Branch, DCBD National Institute of Mental Health, Laboratory of Molecular Biology, LMB		
LAB/BRANCH Surgical Neurology Branch		
SECTION Biochemistry Unit		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Bone marrow transplantation is the treatment of choice for high risk leukemia, aplastic anemia and other immunodeficiency disorders. It is also being used for therapy of other radiation sensitive tumors such as neuroblastoma and for inherited enzyme deficiency disorders. The limiting complication is graft-versus-host disease (GVHD) caused by mature T cells in the donor marrow recognizing histocompatibility differences between donor and host. Studies in animals and humans have shown that removal of mature T cells from the donor marrow while preserving the pluripotent stem cells prevent GVHD. Monoclonal antibodies linked to toxic proteins can specifically kill cells based on cell surface antigen differences. We have developed a panel of T cell selective toxins which kill up to 5 logs of T cells at concentrations non-toxic to human stem cells. We have begun clinical trials of these reagents for 1) prevention of GVHD in MHC matched bone marrow recipients; 2) prevention of GVHD in MHC mismatched BMT. Twenty three patients have now been treated. Ten patients transplanted for high-risk leukemia with major HLA matched sibling marrow are now evaluable. The antibody-ricin conjugate showed no toxicity to the patients. Comparing antibody-toxin treatment with conventional post-transplant methotrexate prophylaxis the hospitalization stay was significantly shorter. Engraftment of donor marrow occurred in all patients and 8 out of 10 showed predominantly donor lymphoid cells 30 days post-transplant. No cases of severe GVHD were observed. Continued clinical trials are underway to increase population size to statistically significant levels to compare GVHD incidence of immunotoxin vs. conventional protocols.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02674-03 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Monoclonal Antibody-Toxin Conjugates for Tumor Therapy in vivo		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Richard J. Youle, Ph.D. Virginia Johnson, Ph.D. Debbie Wilson Jin Fu-Sheng, M.D.	Principal Investigator, SNB, NINCDS Staff Fellow, SNB, NINCDS Bio Lab Technician, SNB, NINCDS Special Volunteer, SNB, NINCDS	
COOPERATING UNITS (if any) Laboratory of Immunology, NIAID Cetus Corporation		
LAB/BRANCH Surgical Neurology Branch		
SECTION Biochemistry Laboratory Unit		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.5	PROFESSIONAL 1.5	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Monoclonal antibodies selectively bind tumor cell differentiating antigens <u>in vitro</u> and <u>in vivo</u>. Natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells so we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells. Two methods of coupling toxic proteins, like ricin to antibodies, have been used to kill antigen positive cells <u>in vitro</u>. Ricin has two subunits, the A subunit blocks protein synthesis when in the cytosol and the B subunit binds galactose groups on all cell surfaces but also facilitates the transport of ricin A chain to the cytosol. 1) Linkage of the ricin A chain to antibodies yields reagents with low non-target toxicity but target cell toxicity too slow for <u>in vivo</u> applications; 2) Linkage of intact ricin to antibodies results in very potent target cell toxicity but the non-target cell killing must be prevented by a ligand which blocks ricin B chain binding to cells. This has limited its application to <u>in vitro</u> situations where 100 mM lactose can block ricin binding. We have succeeded in developing several new approaches to apply immunotoxins <u>in vivo</u>. 1) Cloning of toxins then altering their structure at the gene level to decrease non-target cell toxicity; 2) Chemical modification of ricin to determine the location of the ricin galactose binding site and to possibly improve efficacy of ricin linked to antibodies; 3) Develop new ways to block the non-target cell toxicity of ricin <u>in vivo</u>. We have discovered a monoclonal antibody which blocks the ricin galactose binding site similar to lactose; 4) Intrathecal administration of monoclonal antibodies allows a new way to image brain tumors; 5) Intrathecal administration of immunotoxins for therapy of brain tumors that kill 2 to 5-logs of tumor cells in animal models; and 6) Preparation for clinical trials of human brain tumor patients.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02708-02 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Vascular Permeability Factor Produced by Human Glioma Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Gregory Criscuolo, M.D. Marsha Merrill, Ph.D. Edward H. Oldfield, M.D.	Senior Staff Fellow, Principal Investigator, SNB Staff Fellow, SNB, NINCDS Chief, SNB, NINCDS	
COOPERATING UNITS (if any) n/a		
LAB/BRANCH Surgical Neurology Branch		
SECTION Tumor Biology Unit		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL 2.0	OTHER 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Cerebral edema associated with malignant brain tumors neurologic deficits and increased intracranial pressure, which contribute to the morbidity and mortality of the neoplasm. We have determined that the conditioned medium of glioblastoma-derived cell cultures contains a substance capable of increasing the vascular permeability in a bioassay which measures the induction of capillary permeability in normal skin. The elicitation of this response by conditioned medium correlates with the edema observed clinically in patients from which these tumor cell cultures were derived. This factor appears to be distinct from other known inducers of vascular permeability. We are now in the process of purifying and characterizing this factor. Our long range goal is to better understand the regulation and mechanism of action of this factor.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02707-02 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Role of Insulin and Insulin-like Growth Factors in Glioma Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Marsha Merrill, Ph.D. Nancy Edward Robert Friedman, M.D. Calvin Hawkins	Staff Fellow, Principle Investigator, SNB Biologist, SNB, NINCDS Medical Staff Fellow, SNB, NINCDS Bio Laboratory Technician, SNB, NINCDS	
COOPERATING UNITS (if any) n/a		
LAB/BRANCH Surgical Neurology Branch		
SECTION Tumor Biology Unit		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Insulin and insulin-like growth factor (IGF) receptors have been detected in normal human brain. However, the role of IGFs in the brain is not understood. Although considered to be primarily a fetal growth factor, IGF-II levels continue to be detected at high levels in the CNS of adults, although the functional significance of this observation is unknown. Similarly, what role IGFs may play in brain tumors is unknown. We conduct studies on normal and malignant brain tissue and in cell cultures derived from these tissues to begin to understand the physiologic significance of this family of growth factors in human brain. Our studies demonstrate a high level of IGF-I receptors on low grade astrocytomas, suggesting a role for IGF-I in brain tumor oncogenesis. At least some glioma derived cell lines also produce IGFs as measured by competition in a radioreceptor assay.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02729-01 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Adrenal Medullary Autografts in Parkinsonian Patients		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Edward Oldfield, M.D. Chief, SNB, Principal Investigator, NINCDS Robert J. Plunkett, M.D. Senior Staff Fellow, SNB Kris Bankiewicz, M.D. Visiting Fellow, SNB Jeff Nordin, M.D. Senior Staff Fellow, NCI Hetty DeVroom, R.N. Clinical Nurse, SNB, NINCDS		
COOPERATING UNITS (if any) Surgery Branch, National Cancer Institute		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The behavioral, biochemical, and histological effects of tissues implanted into the brain of mammals is being studied. Rodent and primate models of Parkinson's disease are recipients for dopaminergic tissue implants. Fetal mesencephalic grafts to the striatum have been the most successful for restoration of function in the primates. We have also observed sprouting from non-damaged dopaminergic cells in the implanted animals. This may be the basis of the functional recovery and is now under active investigation. Part of our effort has been to perfect the technical aspects of stereotactic implantation of dispersed cells into brain. We identified the factors which influence the accuracy of cell placement, and will now use this information to implant various dopaminergic cells into the brains of lesioned animals. In addition, we are developing a model of intracerebral pancreatic islet implantation in diabetic rats to facilitate studies of interaction of the peripheral immune system and tissue grafts into the brain.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02727-01 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neural Regulation of Immunity		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard Weber, Ph.D. Agu Pert, Ph.D. Barbara Ikejiri Martha Johnson Kenner C. Rice Senior Staff Fellow, Principal Investigator, SNB Research Pharmacologist, BPB, NIMH Biologist, SNB, NINCDS Bio Lab Technician, SNB, NINCDS Research Chemist, LN, NIADDK		
COOPERATING UNITS (if any) Laboratory of Neuroscience, NIADDK		
LAB/BRANCH Surgical Neurology Branch		
SECTION Neuroimmunology Unit		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.2	PROFESSIONAL: 1.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Initial studies confirm that systemic administration of morphine (50 mg/kg) in male Fisher 344N rats resulted in a suppression of NK cell activity. Intracerebroventricular administrations were also found to produce a dose dependent suppression of NK cell activity. In order to localize the CNS effects of morphine on immune function, rats were implanted with bilateral cannulae guides aimed for various brain structures. One week following surgery the animals received bilateral injections of morphine (5 ug in 1 ul saline) in each structure. Three hours following administration of morphine the rats were sacrificed, spleens removed, and both NK measured. Injections of morphine into the anterior hypothalamus arcuate nucleus, medial amygdala, medial thalamus, and dorsal hippocampus had no significant effect on the parameters of immunocompetence examined, when compared to uninjected animals. Injection of morphine into the periaqueductal gray matter, however, produced a suppression of immune function, when compared to saline injected animals. These findings suggest that the central actions of opiates on immune function are mediated through the PAG. The precise neural mechanisms involved, however, remain to be elucidated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02728-01 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on the Heterogeneity of Drug Delivery During Intracarotid Chemotherapy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen Saris, M.D. Senior Staff Fellow, Principal Investigator, SNB Ron Blasberg, M.D. Donald Wright, M.D. Edward H. Oldfield, M.D. Jonathan Tweed, M.D. Senior Investigator, Department of Nuclear Med. Medical Officer, SNB, NINCDS Chief, SNB, NINCDS Special Volunteer, SNB, NINCDS		
COOPERATING UNITS (if any) Department of Nuclear Medicine		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our objective is to study the physiology of intraarterial infusions in the carotid artery. A major problem with intracarotid infusions in patients with gliomas is focal injury to the eye and brain. Our hypothesis is that these problems are due to poor mixing of the infusate with blood such that some regions of the target tissue get very high concentrations of the drug which results in infarction of tissue. Other areas may receive suboptimal drug delivery resulting in treatment failure. We demonstrated heterogenous delivery of an intraarterially infused isotope in animal models, and are now studying patients. We hope to eliminate this poor mixing with a specifically engineered infusion pump which injects in a pulsatile manner during the slow blood flow phase of diastole. If drug streaming can be eliminated by this infusion technique, it should permit a convenient and practical means of infusion in the clinic. Although we have used this carotid arterial system and the brain and eye as target organs, the principles established should be generally applicable to other arteries and organs.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 NS 02686-03 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunotherapy of Brain Tumors by IL-2 and Activated Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
David Barba, M.D.	Senior Staff Fellow, Principal Investigator, SNB	
Stephen Saris, M.D.	Senior Staff Fellow, Principal Investigator, SNB	
Joseph Alexander, M.D.	Medical Staff Fellow, SNB, NINCDS	
Barbara Ikejiri	Biologist, SNB, NINCDS	
Edward Oldfield, M.D.	Chief, SNB, NINCDS	
Stephen Rosenberg, M.D.	Chief, Surgical Oncology Branch, NCI	
COOPERATING UNITS (if any) Surgical Oncology Branch, NCI		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Culture of brain tumor patient peripheral blood lymphocytes (PBL) with recombinant Interleukin-2 (rIL-2) results in the activation of lymphokine activated killer LAK cells capable of killing brain tumor cells. PBL obtained from brain tumor patients by constant flow cell separators were cultured with IL-2 for three to five days. Cells were tested for their ability to lyse fresh tumor targets. These LAK cells were infused with IL-2 into patients brain tumor cavities, via a previously placed reservoir and cell infusion system. This reservoir system was also used to draw specimens of cells and fluid to test for IL-2, evaluate surviving cell number, and LAK activities. Serial CT scans were used to assess the patients response to treatment. Brain tumor patient's PBL were cultured with IL-2 lysed fresh tumor targets. LAK cells and IL-2 infusions were tolerated in brain tumor patients, but with toxicities, transient or permanent, related to the site of treatment. Six patients received 9 treatments; high levels of IL-2 were found in the cyst fluid during treatment. Cell number and LAK activity decreased with time in the cyst. Radiologic evidence of tumor regression was seen in one of five patients evaluated to date. In addition, by performing lumbar drainage with a small lumbar subarachnoid catheter, we study the kinetics of IL-2 in the CSF, and determine the penetration of IL-2/LAK into the CSF and investigate if there is any effect on the blood-brain barrier. In addition, by performing lumbar drainage with a small lumbar subarachnoid catheter, we study the kinetics of IL-2 in the CSF, and determine the penetration of IL-2/LAK into the CSF and investigate if there is any effect on the blood-brain barrier.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02687-03 SN		
PERIOD COVERED October 1, 1986 through September 31, 1987				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Laboratory Studies for Development of IL-2/Activated Lymphocyte Treatment of BT *				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard J. Weber, Ph.D. Senior Staff Fellow, Principal Investigator, SNB				
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%; vertical-align: top;"> Joseph Alexander, M.D. David Barba, M.D. Gregory Criscuolo, M.D. Barbara Ikejiri Stephen Saris, M.D. </td> <td style="width: 60%; vertical-align: top;"> Medical Staff Fellow, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS Biologist, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS </td> </tr> </table>			Joseph Alexander, M.D. David Barba, M.D. Gregory Criscuolo, M.D. Barbara Ikejiri Stephen Saris, M.D.	Medical Staff Fellow, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS Biologist, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS
Joseph Alexander, M.D. David Barba, M.D. Gregory Criscuolo, M.D. Barbara Ikejiri Stephen Saris, M.D.	Medical Staff Fellow, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS Biologist, SNB, NINCDS Senior Staff Fellow, SNB, NINCDS			
COOPERATING UNITS (if any) n/a				
LAB/BRANCH Surgical Neurology Branch				
SECTION Neuroimmunology Unit				
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892				
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 1.9	OTHER: 0.0		
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Brain tumors are generally resistant to killing by cytotoxic effectors of the immune system including cytotoxic T-lymphocytes (CTL). Incubation of lymphocytes with recombinant interleukin-2 (IL-2), however, generates lymphokine activated killer cells (LAK) endowed with tumoricidal capacity. Rodents inoculated intracranially with autologous glioblastoma cells exhibit increased survival when treated with IL-2. This phenomenon is believed to occur due to the infiltration of tumor by LAK generated <u>in vivo</u>. LAK have been generated <u>in vitro</u> which produce lysis of autologous and allogeneic tumor tissue but not normal PBL. We have compared various routes of administration of IL-2 and LAK to assure optimal delivery of lymphokine and killer cells to obtain a favorable therapeutic effect. Patients receiving IL-2 experience toxic side effects associated with the extravasation of intravascular fluid and the development of a vascular leak syndrome. We have shown that a product of lymphocytes activated <u>in vitro</u> with IL-2 produces a similar phenomenon when tested in a modified "Miles" assay. This substance is macromolecular in nature and is the product of lymphocytes or mononuclear cells. * (Formerly: "Mechanisms of Interleukin-2 Activators of Cytotoxic Lymphocytes")				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02454-07 SN
PERIOD COVERED October 1, 1986 through September 31, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Human Pituitary Tumors *		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Edward Oldfield, M.D. Chief, SNB, Principal Investigator, NINCDS		
COOPERATING UNITS (if any) Developmental Endocrinology Branch, NINCDS Diagnostic Radiology, CC		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We continue to investigate venous samplings of the pituitary venous drainage to aid in the diagnosis and treatment of patients with Cushing's syndrome. Over 150 patients have now received bilateral simultaneous inferior petrosal sinus (IPS) sampling. The results indicate that 1) procedure can be performed successfully in all patients with Cushing's syndrome (successful sampling has been performed in over 99% of the patients in whom it has been attempted); 2) the procedure distinguishes patients with ectopic ACTH secretion from those with pituitary adenomas with greater than 99% accuracy; 3) if one adds CRF stimulation to the diagnostic procedure, a diagnostic accuracy of 100% has been achieved in over 100 patients with Cushing's syndrome; 4) IPS sampling successfully determines which side of the pituitary gland microadenomas reside in patients with Cushing's disease with 85% accuracy; and 5) unilateral inferior petrosal sinus sampling, which is commonly used clinically, is frequently misleading. Repeat transssphenoidal surgery is successful in irradiating the hyper-cortisolism of Cushing's disease in about 60% of patients. This therapy for patients with Cushing's disease after previous pituitary surgery has not previously been examined. The CRF stimulation test and the dexamethasone suppression test are equally accurate in determining and distinguishing patients with Cushing's disease from those with ectopic ACTH secretion in Cushing's syndrome. Both have approximately 10-15% diagnostic inaccuracy, when used alone, in our experience. CT scanning of the pituitary gland is normal in 70% of patients with Cushing's syndrome. *(Formerly: "Biological Studies of Human Pituitary Tumors")		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02696- 03 SN
PERIOD COVERED October 1, 1986 through September 31, 1987 *		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intraarterial Chemotherapy Combined with Extracorporeal Drug Removal		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Edward H. Oldfield, M.D.	Chief, SNB, Principal Investigator, NINCDS	
Dr. Robert Dedrick	Bioengineering and Instrumentation Branch Division of Research Services, NCI	
John Doppman, M.D.	Diagnostic Radiology Department, CC	
COOPERATING UNITS (if any) Bioengineering and Instrumentation Branch, Division of Research Services Diagnostic Radiology Department, Clinical Center		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intra-carotid infusion. Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high con-centration of drug reaches the general circulation, should allow dose escalation to levels which provide increased tumor response. The following have been demonstrated. 1) A pilot study of 4 patients treated with intracarotid BCNU during extracorporeal hemoperfusion of the jugular venous blood demonstrated that the drug exposure of the body could be reduced by 56-87% by channeling the blood extracorporeally at 300 ml/min through a hemoperfusion cartridge for drug extraction. The pharmacokinetic advantage (ratio of brain exposure to body exposure) compared to intravenous infusion of the same dose was 21-55:1; 2) Drug streaming during intracarotid delivery results in mal-distribution of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas receive minimal drug. Drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU; 3) A dye dilution study of intracarotid infusion of indocyanine green demonstrated that the recovery of substances from the jugular blood after intracarotid infusion is linearly related to the rate of aspiration of the blood from the jugular blood. This suggests that the amount of an injected drug which can be removed from blood before the blood is returned to the body is dependent on the rate at which blood is withdrawn from the jugular vein; 4) Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion of cis-platinum resulted in tumor exposures 5-15 flow greater than the exposure of the remainder for the body. *This project is terminated 9/87.		

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